INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the Chicago Section[1]
May 23, 1929

B. ORBAN, M.D., *Editor*

Meeting held at the West Side Student Y. M. C. A. Building, Wood and Congress Streets, Chicago, May 23, 1929. Chairman: Edward H. Hatton, B. L., M. D., President of the Section.

Scientific program

I. The relative toxicity of local anesthetics on white mice. *Harold T. Dailey*, D.D. S., and *H. C. Benedict*, B.A., M.A..... 609
II. An item in the study of the dental lymphatics. *Frederick B. Noyes*, B.S., D.D.S., D.Sc., and *Robert L. Ladd*, M.D..... 609
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Abstracts

I. THE RELATIVE TOXICITY OF LOCAL ANESTHETICS ON WHITE MICE

Harold T. Dailey, D. D. S., and H. C. Benedict, B. A., M.A., Northwestern University Dental School, Chicago, Ill.

The report related to an anesthetic (name not mentioned, but called "x") that induces relatively slight toxic effects on white mice, and a much faster action than any other. The drug was compared with other anesthetics used, and was found to be much superior to them. Clinical experiments on patients were suggestive of similar results. A scientific report will soon be published.

II. AN ITEM IN THE STUDY OF THE DENTAL LYMPHATICS

Frederick B. Noyes, B. S., D. D. S., D. Sc., and Robert L. Ladd, M. D., College of Dentistry, University of Illinois, Chicago, Ill.

In this work, ferric ammonium citrate was forced through the dentine into the dental pulp, and found in the lymphatic channels of

[1] The last preceding series of abstracts of the proceedings of the Chicago Section was published in the *Journal of Dental Research*: 1929, ix, p. 79.

the first lymphatic gland of the submaxillary group in dogs. A pressure of from twelve to eighteen inches of mercury was used and a special apparatus designed, which will be described in full in the paper as read before the New York State Dental Society, on May 15, 1929.

III. EARLY HUMAN TOOTH DEVELOPMENT, WITH SPECIAL REFERENCE TO THE RELATIONSHIP BETWEEN THE DENTAL LAMINA AND THE LIP-FURROW BAND[2]

Isaac Schour, B.S., D.D.S., M.S., College of Dentistry, University of Illinois, Chicago, Ill.

This study is based chiefly on uninterrupted serial sections taken from the human-embryological collection of the Department of Anatomy of the University of Chicago. The histological findings of the more recent investigators of human tooth development are corroborated for the most part with original photographs. The relationship between the dental lamina and the lip-furrow band is found to vary according to the stage of development, and also according to the location respecting the anterior or posterior direction and the upper or lower jaw. These variations can be explained by the workings of the laws of unequal growth and interdependence of developmental processes, with the resultant shifting and crowding of relationships.

IV. CONTRIBUTION TO THE HISTOLOGY OF THE PULP AND PERIODONTAL MEMBRANE, WITH SPECIAL REFERENCE TO THE CELLS OF DEFENSE IN THESE TISSUES

B. Orban, M.D., Chicago College of Dental Surgery, Loyola University, Chicago, Ill.

Lantern slides were presented, showing the presence of histiocytes, undifferentiated mesenchyme cells, and ameboid wandering cells in the normal human pulp and periodontal membrane. On the basis of a specimen of chronic abscess at the apex of a bicuspid, the transformation of these cells into "polyblasts" was shown. The polyblasts of Maximow are phagocytic, mononuclear, wandering cells in the field of inflammation.

[2] Published in full in the *Journal of Dental Research*, 1929, ix, p. 699 (October).

PREVENTIVE DENTISTRY[1]

A Symposium

WILLIAM DWIGHT TRACY, D.D.S., F.A.C.D.; HAROLD J. LEONARD, D.D.S., B.A.; FRANK A. DELABARRE, D.D.S., M.D., F.A.C.D.; and SHERMAN L. DAVIS, M.D., A.M., Ph.D.

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I. OPERATIVE PROCEDURES AS RELATED TO THE PREVENTION OF DENTAL CARIES

William Dwight Tracy, D.D.S., F.A.C.D., New York City

This symposium on the prevention of dental diseases and abnormalities, which has been arranged by our executive committee, is another of the many evidences that prevention as a principle of practice is prominent in the minds of dental practitioners everywhere. It is perhaps safe to say that it is the dominant thought in the minds of all those engaged in the healing art, and finds expression throughout its many ramifications. In support of this statement, witness the many organizations established for the purpose of education and prevention, such as the Federal Health Bureau, the National Tuberculosis Association, the New York Tuberculosis and Health Association, the Society for the Prevention of Cancer, the Mental Hygiene Association, the Gorgas Memorial and many others of like purpose. And in the schools of dentistry we find now that special attention is given to preventive dentistry with a view to inculcating in the minds of students the vital necessity of recognizing in their incipiency all those departures from the normal which if neglected lead to dental diseases of various types.

[1] Presented at a meeting of the New York Academy of Dentistry, Commodore Hotel, New York City, February 21, 1929.

No student who has not become familiar with the beautiful clinical pictures of normality, or nature at her best, is equipped to recognize in their early stages those dyscrasias which when neglected result in permanent injury to the teeth and their investing tissues. While there are certain procedures to which the operative dentist may resort to inhibit the progress of dental diseases after they are found to be present, the most effective measure for keeping the teeth, the gums, and the oral mucosa from the invasion of disease is undoubtedly periodical prophylactic treatment. Even when prophylaxis is administered at frequent and regular intervals, and the patient gives complete and intelligent coöperation, there seems to be no certainty of freedom from decalcification of dental enamel and no assurance that well placed fillings already in the teeth may not suffer from secondary caries. We think and we hear a great deal about prevention of dental diseases and we must pursue our ideal with concentrated effort and intelligent zeal; but it is the observation of the writer that the cases in which we actually reach the goal are a very small percentage of the entire number of the patients treated. This may sound pessimistic but it is not intended to be so, and we should be encouraged by the thought that, if we had not made use of the preventive measures which are at our disposal, our patients in all probability would be in a much worse condition dentally.

There still seems to be much confusion in the minds of those who lead in dental thought concerning the incidence of caries. With some the idea seems to prevail that the teeth yield to bacterial invasion because the enamel is lacking in what they term normal vital resistance, and that this so-called vital resistance may be built up or improved by specific forms of diet. With others there seems to be a firm conviction that dental enamel once formed is not under any conditions subject to nutritional change, either for better or worse, and that the incidence of decalcification of enamel and subsequent progress of caries is due to the formation of dental plaque and the presence in the oral cavity of the Bacillus acidophilus in excessive numbers. In other fields of health study and service it has been found necessary to learn the cause of a human malady before effective measures could be successfully applied for its prevention. With confusion in the minds of dental leaders as to the reasons for defective dental development

and as to the biological characteristics of enamel, it is natural that efforts at prevention should also be at variance and somewhat confused. It is hoped, therefore, that science will in due course shed more light on some of these problems, to make it possible more intelligently to pursue our activities aimed at prevention. Conditions being as they are, and certain departures from the normal being practically omnipresent in the human teeth, we have no recourse except to apply those measures that have proved meritorious in preventing congenital defects from becoming worse, and in reducing the susceptibility of the teeth to acquired defects of environmental origin.

The study now being made by Professor Bunting and his associates in regard to the correlation existing between decalcification of enamel and the B. acidophilus content of the oral flora is intensely interesting, and seems to give promise of furnishing a tangible means of reducing the tendency to enamel decalcification by waging direct war upon that organism. Normal diet, or that selection of food stuffs which will build up and maintain good bodily health, is desirable from any view point, but from the dental point of view the elimination of soft, sticky, carbohydrate, sweet, and highly refined foods is desirable; and the addition of fresh vegetables, both root and leafy, whole-grain breads or resistant texture to induce mastication, and fresh fruits to aid in a natural cleansing of the teeth and stimulation of oral secretions, is essential. Such a diet may be beneficial to the tooth enamel through nutritional and metabolic processes, but we know that its influence is beneficial locally to the tooth surfaces, and that its influence upon the dental environment is beneficent.

The procedures aimed at the prevention of dental caries, and which we may correctly term as operative, naturally fall into two groups: (a) those used in caring for the deciduous teeth, and (b) those applied in the care of the permanent denture.

Under (a), the operative procedures used to prevent dental caries in deciduous teeth, must be mentioned early and frequent examination, periodical prophylaxis, the application of ammoniacal silver nitrate and formalin to pits and fissures in the molars, and prophylactic odontotomy where indicated. In a large percentage of children, where the silver nitrate is applied to pits and fissures with a view to postponing the day when small fillings may be necessary, it is frequently

found, by repeating the applications semi-annually, that teeth may be carried to the time of normal exfoliation without need for the insertion of fillings. With the less fortunate children, who develop cavities on the proximal surfaces of the deciduous molars, it is frequently desirable to extend the cavity preparation to include these uncertain defects. It is assumed, of course, that while these operative measures as applied by the dentist at stated intervals are being carried out, the child's home routine in regard to the care of the mouth and teeth is also being religiously pursued, as it is only through this type of coöperation that the best results are obtained.

Under (b), the operative procedures that are applied in the care of the permanent dentures, we will assume that the patient is about fourteen years of age and has acquired all the teeth of the permanent denture with the exception of the four third molars; we will assume also that the case has been continuously under our care since the eruption of the deciduous teeth, which were all retained without accident up to the time of their normal exfoliation. When the first permanent molars arrived, they were thoroughly dried and carefully examined for evidence of enamel defects. If very irregular and presenting many convolutions upon the occlusal surfaces, a small mix of oxyphosphate of copper was made and placed upon the tooth. Over this a piece of tin foil about three quarters of an inch square was placed and the mass of cement pressed down (or up) with the ball of the finger, thus spreading the material and crowding it into all the little irregularities of the tooth surface. When set, the surplus was removed and occlusion adjusted. Protections of this sort frequently last for a year, when the application may be repeated if desirable. Where nothing is done to these teeth the first year after eruption, it may be decided that certain pits and fissures should receive attention the second year. With these it has been common practice for many years to cut them out with small burs, fill them with tiny plugs of pink base-plate gutta-percha, which would offer adequate protection up to thirteen or fourteen years of age, when they were replaced with small fillings of non-cohesive gold foil.

Systematic application of the Hyatt method of prophylactic odontotomy, using amalgam as a filling material, is a safe and sure way of protecting the occlusal surfaces of all molars and bicuspids from the

invasion of caries. Such fillings, when the amalgam is properly manipulated, inserted, and polished, give every possible assurance of permanency. Dr. R. Ottolengui is recommending silicate cement fillings for the cavities produced in the practice of prophylactic odontotomy. His advocacy of these fillings is based on the statement that in the mouths of young children they are simple to manage and quickly inserted. By a puddling movement with small points the silicate cement is carried into the cavities, insuring thorough placement. After insertion, and before the material has set, he uses a small brilliantly polished burnisher of pineapple shape, which carries the filling material down into the fissures and gives it a properly groved surface contour. Dr. Ottolengui states that fillings of this type are practically invisible and that, after five years of use, they are found to be perfectly intact.

It would seem, then, that there are at least three ways of managing pits and fissures occurring upon the first permanent molar: (a) to stop them with small gutta-percha fillings, and to replace these with fillings of gold foil when the child is older and better able to coöperate; (b) the Hyatt method of prophylactic odontotomy, using amalgam as the filling material; and (c) the use of silicate cement as suggested by Dr. Ottolengui. Some of us, who were schooled to believe that there was no better filling than gold foil for cavities of the type under consideration, and who still feel that it is the best material, prefer to adhere to its use as outlined above. However, it is well proved that high grade amalgam in these cavities, when properly used, has all those qualifications which enable us to classify it as a permanent filling. The silicate cements have been a source of so much disappointment to us for so many years that we naturally regard them with some skepticism, even when used in pits and fissures.

In seeking to prevent caries in the six anterior teeth, and in the bicuspids, all we can do as far as operative procedure is concerned is to watch over them carefully, and with fine strips polish the proximal surfaces where any suspicious area indicating decalcification of enamel is observed. Baselar pits in the maxillary laterals, and small defects on the palatal aspect of the centrals as well, can usually be filled with gold foil as soon as discovered after eruption. Defective sulci and pits in all bicuspids naturally receive the same treatment as that

used for similar defects on the molars. It is quite essential that the dentist should see his growing patient immediately after the exfoliation of the second temporary molars, because at this time he has his best opportunity to examine, and if necessary to treat, the anterior proximal surface of the first permanent molars. Frequently a small discolored spot is observed, which can be disked off and left with a highly polished surface that is easily produced with cuttle fish or other types of extremely fine disks. This treatment may avert the necessity of inserting a filling on this surface at a later date. If the enamel is actually involved with deep decalcification, it then becomes necessary to establish a properly shaped cavity, and for filling cavities in this location gold foil would be the writer's choice.

The second molars have not, up to this point, been specifically mentioned, but whatever method is employed in dealing with defects in the first molars is equally applicable in caring for the second molar. When all the permanent teeth are in place, a semi-annual x-ray survey of the teeth should be established as a definite routine, for it has been found that even a most careful scrutiny of the teeth, using the finest exploring instruments available, does not always enable the operator to locate obscure caries where the enamel has been penetrated at the point of proximal contact. Radiograms frequently disclose defects on the proximal surfaces that could in no other way be discovered, and are therefore regarded as an essential means to prevent the unwitting neglect of caries already established.

In the adult patient, there are certain conditions found upon the teeth which may be controlled by proper treatment. For example, decalcification of enamel at the cervical portion of the anterior teeth, if discovered in its early stages, may be polished out with fine stones and disks. This surface is then given a high lustre by using tin oxide with rubber, or moose hide polishers mounted upon a mandrel and used in a dental engine. Surfaces thus polished, if properly cared for by the patient and dentist, may never need fillings. Another situation occurs where recession of the gum has taken place laying the neck of the tooth bare above the enamel line. If the tooth structure has really suffered disintegration, it must of course be converted into a properly shaped cavity and some form of cervical filling inserted, but if the surface will still permit of polishing, that should be done in

the manner outlined above, after which 40 percent formalin solution may be applied and left for a minute and a half or two minutes. This treatment is likely to cause a brief painful reaction, but it seems to have a salutary influence upon the exposed structure, reducing the sensitiveness and apparently rendering the exposed surface more resistant to the influence of caries. In locations not exposed to view, silver nitrate is used for the same purpose. It causes discoloration of the area to which it is applied, but seems to be more lasting in its effect.

In closing this brief paper it seems fitting to quote from an admirable essay by Frederick W. Schultz, M.D., which was presented before the Section on Preventive Medicine at the meeting of the American Dental Association, August, 1928. The quotation is as follows:

"Dentistry is triumphant today in the art of mechanical repair, but it is still baffled by the mystery of the factors which lie beneath the dental disorder and which duly understood would prevent its occurrence. We know that a tooth perfectly formed and kept fully intact by natural or artificial means may remain healthy and in function for a lifetime under ordinary conditions of use and even regardless of oral hygiene. The fact rather confirms the opinion now generally held that the secret of the prevention of dental abnormality really lies hidden in the constructive processes of normal dentition rather than in the conditions of superficial oral hygiene, important as the latter doubtless is in removing extrinsic hostile agents or in neutralizing dentally destructive influences."

II. THE PREVENTION OF PERIODONTAL LESIONS

Harold J. Leonard, D.D.S., B.A., New York City

It is sometimes possible to cure disease by treating symptoms with empirical methods without understanding fully the nature of the disease or its etiology, but the sure prevention of disease in the main requires very accurate knowledge of its causes and pathogenesis. The reason why we cannot prevent dental caries is because we do not know exactly the causes and pathogenesis of susceptibility to the disease. Similarly, we cannot completely prevent periodontoclasia because some of the causative factors are still unknown. However, with a clear understanding of periodontoclasia on the part of the dentist,

and intelligent coöperation on the part of the patients, there are but few cases in which the disease cannot be prevented.

The simplest explanation of causes of periodontoclasia includes consideration of three factors, which are, (a) bacterial invasion from the tooth surfaces, (b) slowly acting mechanical injuries, (c) and lowered vitality in the periodontal-tissue cells. Each of these factors is capable of subdivision, and usually more than one acts at a time; but if these three be held in mind at all times, it makes the consideration of this subject comparatively simple.

Bacteria are of course present in the mouth from a few hours after birth to death. As the teeth erupt the bacteria multiply greatly, owing to the increased number of lodging places on the enamel, between the teeth, and in the gingival crevices. In an unbrushed mouth the scrapings of material from the tooth surface may contain as many as six to eight hundred million bacteria per milligram, according to Kligler and Gies (1). In a well kept mouth the numbers may be one hundredth part of this. According to Gottlieb (2) there are very few sections of gingival tissue that do not show microscopic evidence of bacterial invasion at the base of the gingival crevice. He had sections from one case in which the teeth had been exceptionally well brushed and there were no signs of gingival invasion. In persons of greatly functioning and heavily developed teeth and jaws, like the Esquimaux, in which mechanical factors and cell vitality favor the periodontium, a gradual, even recession frequently occurs according to Waugh (3), accompanied by heavy deposits of salivary calculus. This is evidently a periodontoclasia due solely to bacterial invasion, the heavy bacterial masses that accompany the calculus formation causing a slow disintegration of the gingival tissues, and leading finally to marked loss of alveolar process and periodontal attachment. The exudate from the base of an infected gingival crevice gives rise to deposits of serumal calculus, which are highly irritating in themselves owing to their rough surfaces and bacterial content. Salivary calculus is irritating for the same reason. Rough enamel surfaces or faulty-filling margins act as irritants in the same way by serving as lodging places for bacterial growth.

The obvious means of preventing this type of periodontoclasia is to keep the teeth clean by brushing. However, the problem is not

quite so simple as it seems. G. V. Black (4) discovered that, if he ate heartily of certain types of food and rested after eating, there was a great secretion of protein material in his saliva that coated his teeth. He investigated the subject by means of a microscope cover-glass inserted in a little frame in his upper denture opposite Stenson's duct. By removing the cover glass at intervals, staining, and examining under the microscope, he studied the nature of the material. He concluded that this was the mechanism by which salivary calculus forms. This protein material, combined with lime salts, is deposited on the teeth principally near the outlets of the salivary ducts. An intense bacterial activity goes on in this material, until the organic part is used up, leaving a residue of lime salts on the teeth. This mechanism undoubtedly accounts for the heavy calculus deposits in those that habitually over eat, and also for the furry feeling of the teeth following over indulgence. The active bacterial action is responsible for the gingivitis, superficial periodontoclasia, and generalized recession.

The gingiva is a tissue wonderfully adapted to a special use, which is to serve as a protective cushion for the supporting bone against the mechanical injuries incident to a rough mixed diet and animal warfare. Under primitive conditions, where the teeth are used as a means of preparing food, as weapons of offense and defense, and even as tools for other purposes, the teeth get much use and are well developed. The surrounding bone and pericementum are well developed and well supplied with blood because of function, and the gingiva is dense and heavy with an epithelium containing deeply penetrating papillae. Under primitive conditions food is usually scarce and of low caloric value. The net result is a gingiva of high resistance, teeth kept clean mechanically by the cleansing action of coarse food, and but little tendency for deposits to form on the teeth from excess of food products in the saliva. There is little tendency to bacterial growth on the teeth or to infection of the gingiva. Under modern conditions, however, many factors tend to reverse this condition. The teeth are not well developed, but are frequently narrow with comparatively little bulge of enamel to protect the marginal gingiva. The teeth may be crowded and irregularly placed, so as to leave areas where deposits may be protected. The alveolar bone and overlying gingiva usually

are thin, owing to lack of functional development. The periodontal tissues are not well nourished, owing to lack of vigorous function. The teeth are not naturally cleansed by the action of coarse food and vigorous chewing. As a general thing excesses of food are eaten, leading to salivary deposits and heavy bacterial growth on the teeth.

The prevention of periodontoclasia involves many things. In the first place it requires a well developed set of teeth and periodontal structures. There are of course hereditary factors here which are as yet beyond our comprehension, but there is reason to think that much can be done by efforts for functional development and the creation of proper dietary habits. By vigorous functioning of the jaws from the time of birth and of the teeth after eruption, together with proper balancing of dietary and other factors to promote the most perfect bone formation, some experimental results indicate that a much better formation of the periodontal structures may be expected than if these factors are neglected. More will be said of this matter of formation later, in connection with the prevention of traumatic occlusion and of low cell-vitality.

The prevention of bacterial growth on the teeth is not solely a matter of having well developed teeth and periodontal structures. Esquimaux possess these but also have filthy teeth. The difficulty with them is probably a lack of fibrous self cleansing food, and of eating too much at certain periods. It is undoubtedly true that they are frequently starved, but when they do get food they may eat it in excessive amounts. The diet for many of them is exclusively meat for months at a time. For roughage, according to Steffanson (5), they eat fish heads, the cartilaginous and bony parts of which seem to become only partially digested. Such a diet leaves the teeth uncleansed, and promotes the salivary secretion of material that deposits on the teeth and leads to intensive bacterial growth against the gingiva. The result is the superficial periodontoclasia and generalized, slow, horizontal recession previously mentioned. American diets have much the same faults in that they are not self cleansing and frequently lead to deposits on the teeth. Prevention involves moderation in eating, regularity of eating habits, use ot fibrous cleansing foods like acid fibrous fruits and vegetables, and a limitation of foods that tend to produce coatings on the teeth after eating. In addi-

tion to these, is the careful artificial cleansing of the teeth with the tooth brush.

Having discussed the bacterial factor as a cause of periodontoclasia, together with the preventive measures to combat it, let us turn to another, perhaps equally, important cause of disorganization of the investing tissues; namely, frequently repeated mechanical injury. One type of such mechanical injury, which at the same time is associated with bacterial irritation, is the impaction of food into the interdental tissue owing to faulty contact points, occlusal wear, twisted teeth, or separations. Food fibers that impinge on the interdental papilla cause a pressure atrophy leading to recession. But more important than the pressure is the bacterial irritation from the putrefying organic matter, causing deep inflammation and loss of interproximal bone. The tendency for fibers of food to wedge between the teeth is aggravated by the tooth-pick habit, fortunately now less common than formerly. The rough wooden tooth-pick, stabbed into the interproximal gingiva, tends to cause injury and inflammation of the tissue, which further separates the teeth and maintains the unhealthy condition. The smooth, round, pointed tooth-pick, properly used as a massaging instrument for the interproximal gingiva as advocated by Edmands (6), may have a very useful function, but the use of the ordinary tooth-pick in the common way is a thoroughly pernicious habit. The prevention of periodontoclasia, as caused by food impaction, is a matter first of proper operative treatment of tooth surfaces where defects exist, so that the wedging of food is no longer likely or frequent. Where food fibers wedge, they should be removed by means of dental floss, which can be used without causing injury to the interdental gingiva. In cases of recession of the interdental gingiva, dental floss should be used as a routine to remove debris and sticky accretions, which it is practically impossible for brush bristles to dislodge.

A more purely mechanical irritant is the so-called traumatic occlusion. This is a condition of maladjustment of the teeth to each other, in which they are subjected to stresses greater than the supporting structures can withstand or from a different direction. The causes of traumatic occlusion are largely the shifting of teeth because of the loss of some supporting tooth, or to uneven wear of

the teeth, or other pathological condition or operative interference that alters the relations of the teeth. In considering this factor we must study the principles of bone development and formation as established by researches on bone in other parts of the body. We may well pool our experience as periodontists with that of students of bone metabolism in other parts of the body, for periodontia involves this matter essentially as does orthopedic surgery.

According to Jansen (7) the general outer form of bone, that produced by periosteum, is determined in the main by heredity, and it is only partly modified by function. On the other hand the inner portion, the cancellous part, is altogether dependent on function. Plates of bone are built up in the direction of pressure stresses, and are torn down and rebuilt as stress directions change. With gradual increase in stress more and more bony plates are laid down until the bone becomes a solid mass. If the stress becomes too great, resorption occurs. If the stress is removed entirely, resorption gradually occurs. The physiologic points at which overstress or understress causes bone absorption are greatly modified by metabolism, bone absorption occurring much more quickly if bone metabolism is faulty. If, after bone has formed, pressure comes from a direction markedly unlike that for which it has been fitted, absorption occurs very easily. Thus an aortic aneurism may cause absorption, on the anterior surface of vertebrae, from a lateral pressure that cannot possibly be more than one eighth that borne by the same vertebrae in the vertical direction, to which they are adapted.

Applying these facts to the alveolar bone supporting the teeth, we find that bone is built up at the time of tooth eruption to resist the stresses to which the teeth are subjected. MacMillan (8) has shown that the bony plates of the cancellous bone arrange themselves at the root apices more or less radially to resist the intrusive force of mastication. In persons or races in which mastication is heavy, the bone of the alveolar process is thick and the bony plates around the teeth numerous. If function is lost on a tooth, the radial bony plates at the apices are gradually resorbed. If considerable stress be now again placed on the tooth, it will no longer tolerate what it once did, but gets sore and rapid bone absorption

occurs. If a tooth is lost from the arch, the remaining teeth tend to shift in their positions, tipping over into the space. The alveolar supporting bone then receives stress from the side, very different from that to which it was adapted at the time of tooth eruption. The bone may respond by building in new plates to resist the different stress; or, if the latter is great and rapidly changes, resorption occurs. If bone metabolism is poor, resorption tends to occur much more readily and rapidly. The loss of bone in consequence of such altered stresses is a very large phase of periodontoclasia.

Prevention of traumatic periodontoclasia would consist, first, in building heavy bone about the teeth by heavy mastication during the developmental period of the teeth and jaws; second, keeping the teeth in their original masticatory function by replacing lost teeth and maintaining contact points and functional occlusal surfaces; and third, maintaining the bone metabolism at the highest possible point by proper dietetic measures so far as they are known. At present the evidence points to a great decrease in the amount of sugar, white flour, and muscle meat in the dietary, and a great increase in the amount of vegetables and fruit, especially raw leafy vegetables and raw fruit, animal organs and milk, together with sufficient exposure to sunlight, as the best means of insuring good bone metabolism. Orthodontic procedures, by which teeth with no function or distorted function are brought into harmonious relation during the time when alveolar bone is in process of development, are of course an important preventive measure. Orthodontic procedures are of great use in correcting retarded growth, and developing the internal structure, of the bone. It must however be borne in mind that the main bone outlines are laid down by heredity, and bone development much beyond these limitations must not be expected. Efforts in excess of this are liable to bring the roots out through the alveolar process, where the surfaces may remain permanently uncovered by bone, and necessarily much weakened as to stress and to possible infection and recession of the investing soft tissue.

The third general causative factor in periodontoclasia, namely lowered vitality of the periodontal tissue-cells, has already been somewhat considered in discussing resistance to bacterial invasion,

and bone resistance to changing stresses. The health of the tissue cells of an organ is dependent not only on the purity and nutritive elements in the blood, but also on the amount of blood circulated in that organ. Tissue cells elaborate their own poisons, which must be continually washed away and eliminated by a vigorous flow of the blood. The gingival and periodontal tissues are particularly subject to faulty circulation, stasis and congestion, owing partly to infectious processes that cause chronic inflammations and congestions in the gingiva, partly to lack of massage from the heavy chewing of coarse food, and partly to atrophy of some of the supporting tissues caused by the crowding together of the teeth and roots. A sluggish blood flow is undoubtedly responsible for very much of the lowered resistance of the gingival and periodontal tissues to infection. The prevention of low resistance from this cause depends on artificial massage. If congestion and disease are not already present, the tooth-brush massage as given by the interdental brushing technic, first advocated by Barnes (9), and later with modifications by Charters (10), Stillman (11), and others, is sufficient for the purpose. If congestion is already present, the brush massage may need to be supplemented with a deep finger-massage. Low cell-vitality is not always a local matter, and its prevention and treatment cannot be accomplished by local means alone.

Systemic disease and lowered vitality may first manifest themselves in the periodontal tissues. In these tissues infection is present, bacteria are always invading; and unless the disease is progressing, are being repelled by the tissue cells. A balance of forces exists, which is broken when the vitality of the defensive cells is lessened by toxic products in the blood. Diabetes, nephritis, tuberculosis, syphilis, worry, overwork, influenza, all interfere with cell metabolism and predispose to periodontoclasia. They probably do it in two ways, first by furnishing toxic agents, and second by interfering with the normal metabolism of the body. Probably the mineral metabolism is the source of trouble. As Davis (12) pointed out last year, milch cows in calcium equilibrium (that is, giving off exactly the same amount of calcium as they are receiving) can easily be made to give off more than they receive by a mild disturbing

factor in the stable. It is reasonable to suppose that in modern man, whose calcium in the diet is close to the border line of deficiency and whose calcium-activating vitamins are also likely to be low, the calcium is frequently on the negative side, on account of nervous work, worry, and systemic disease. Howe (13), Grieves (14), and others, have caused periodontoclasia in white rats and monkeys, which are normally immune, by feeding diets slightly deficient in lime and in one of the activating vitamins.

In this connection an experience of a friend of mine, a Hindu student is interesting. He came to this country with no dental disease so far as he knew, after living all his life on the usual Hindu vegetarian dietary. A year or two of typical American farm dietary left him with some dental decays, and all the symptoms of periodontoclasia, bleeding gums, slightly loosened and sore teeth. He and some other Hindu students then combined in their living quarters, and prepared their own food in the usual Hindu manner, after which his gums stopped bleeding, and the teeth tightened up, and lost their tenderness without any local treatment whatever. The reason for the development of disease in this case was undoubtedly dietetic. Because he was unadjusted to the farm-hand diet, it probably affected him more adversely than it would if he had been brought up on it. The diet undoubtedly was unbalanced—excessive in carbohydrate, protein, and fat (that is energy foods); and deficient in cellulose, lime, and vitamins. Such a diet would affect the periodontal cells in three ways: first, by depriving them of certain organic food factors essential to their vitality and resistance; second, by poisoning them with toxic products from excessive bacterial putrefaction in the colon; and third, by interfering with the lime or calcium metabolism of the gingivo-periodontal cells and alveolar bone. All of these faults would occur in addition to the local irritation and infection from soft and calculary accretions on the teeth, which usually accompany such a diet.

Many of the ideas here expressed in regard to causes of periodontal disease and its prevention are still unproven. Nothing is more important in the field of preventive dentistry than researches to ascertain the exact relations between these dental diseases—periodontoclasia, susceptibility to dental caries, and deficient

alveolar development—and the systemic causes and factors bringing them about. I have indicated the lines that present evidence indicates preventive measures should take. The evidence is not all in, and present evidence may be overthrown tomorrow.

In conclusion, let me summarize the preventive measures with which there is reason to think periodontoclasia may be minimized if not prevented altogether (a–d): (a) Most perfect development possible of the teeth, jaws, and periodontal structures, by vigorous functioning from earliest childhood and by dietetic care calculated to promote the most perfect bone formation. Such dietetic care should start in the mother before pregnancy. The functional development may be obtained by eight or nine months of breast feeding, and by the use of foods requiring tearing and hard biting as soon as the teeth are erupted. The dietetic element may be obtained at first from normal mother's milk; later from unpasteurized (certified) cow's milk, pure butter, acid fruits, raw leafy vegetables, whole-grain cereals, eggs, and the glandular organs of animals. These should constitute the bulk of the diet, excepting where individual conditions make modification necessary. It does not seem to have been determined whether the addition of calcium salts to the diet aids greatly or not. The diet should be moderate in amount and taken at regular periods. Abundant exposure to sunlight seems to be an aid in the normal metabolism of bone.

(b) The teeth must be kept clean and the gingiva massaged. This includes the habitual use of the tooth brush in an effective manner. The interdental brushing technic as described by Charters (10) is, in my opinion, the most effective for the purpose, especially for adults. A less technical method for children may do for a time, but should be changed as soon as the child is old enough, in order to avoid recessions and cervical abrasions from cross-wise brushing. Faults in tooth structure and arrangement that predispose to bacterial deposits on the teeth, against the gingiva, should be corrected by operative and orthodontic interference. Salivary or serumal calculus is very irritating, and should be removed wherever it has formed.

(c) Traumatic occlusion must be corrected before occlusal trauma has developed. Spaces left from extractions into which other teeth

may shift should be bridged. All the knowledge available about occlusion mechanics should be brought to bear to keep the teeth adjusted to each other in a manner compatible with the bony development about them.

(d) The resistance of the bone to pressure stresses, and of the periodontal cells to infection, is greatly dependent on general metabolism and vitality. The prevention of periodontoclasia, aside from its local aspects, is largely a matter of maintaining systemic vitality and resistance. Especially those dietetic factors that affect the bone are important. Systemic diseases, also worry and nervous overstrain, poison the tissue cells and disturb the metabolic balance. The prevention of most types of periodontal disease is probably as much a matter of bone metabolism as is the prevention of rickets, and is as intimately related to systemic health and hygiene as that of any of the other diseases in the field of medicine.

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III. ORTHODONTIC ABNORMALITIES AS THEY RELATE TO PREVENTIVE DENTISTRY

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The relation between preventive dentistry and orthodontia is very close, since the object of each endeavor is oral health for the sake of general health through the maintenance of oral function. Oral function is complex, inasmuch as the same sets of muscles are concerned in the performance of several distinct functions, such as mastication, deglutition, speech, expression, and also breathing in a passive sense. Disturbance of any of these functional activities adversely affects the others. Study of the etiology of any function must include an estimate of the efficiency of all these functions. Malocclusion is a great disturber of function; complicates preventive dentistry by making the teeth more susceptible to caries, and more difficult to keep clean; and is one of the causative factors in pyorrhea. Preventive dentistry aims to maintain the integrity of the parts, while orthodontia is concerned with the proper arrangements and relationship of the parts. The orthodontist will gladly coöperate with the dentist, and by official resolutions has asked his coöperation in return. Such coöperation is essential to each, since the efforts of either cannot be wholly successful without the aid of the other. The orthodontist rarely sees his cases for the first time before the age of 5, 6, or 7, and the scheme of preventive dentistry as outlined and accepted, calls for the beginning of dental care at the age of 2 or 3. The dentist has the opportunity in those early years to do many things that will help the orthodontist.

The causes of malocclusion are many and imperfectly understood, and may be roughly classified as (a) *basic*, under which is included heredity and organic or functional disturbances that affect the body as a whole, such as the deficiency diseases or ductless-gland involvements; and (b) *contributory*, such as posture, habits, lack of exercise, loss of teeth, etc. Removal of the exciting causes is the first logical step in treatment. The dentist can help by being on the watch for, and detecting the presence of, the causes of malocclusion. The following is only a partial list of some of the more important and more frequent causes that the orthodontist finds in his cases.

There are two items, however, which should be given priority as being of great importance to every one concerned. First is the behavior of the child. Neither the dentist nor the orthodontist can do successful or commendable work if the child is not educated to be a good patient. The whole problem of psychological approach for the professional man is to get down to the level of the child's interests and mental capacity. The second is education in personal hygiene. This work can and should be done by the dentist as a preliminary to his own work. Inasmuch as our efforts can be justly classified as a true health service, a case history should be started at the time of the child's first professional contact. It should include not only the routine examination from the purely dental standpoint, but a consideration of the presence or absence of the common attributes of health, such as the height-weight index, posture, habits of living, color of the skin, expression of the face and eyes, mental attitude of the child, etc. This case history should be supplemented by a medical history whenever indicated by an evident lack of physical tone and vigor. X-rays should be included in order to determine as early as possible the presence of any abnormal conditions such as missing or supernumerary teeth. If, at the time of reference of a case to an orthodontist, such a case history and also a series of models taken in successive years from infancy were available, they would be of the greatest help in studying the previous history and the course of development and eruption of the teeth. With their aid it would be entirely possible for the orthodontist to make an immediate diagnosis as to the trend of development, and to start his work at once without putting the child under observation for a period in order to determine that point.

There are two etiologic factors, either directly causative or contributory, which occur in early childhood and should be noted and controlled by the dentist. The first of these is a group of faulty habits. It is unquestionably easier to eliminate a habit in the early stages of formation, than after it has been in existence for several years. Of all such habits, mouth breathing is by far the most pernicious, and always has an adverse effect on the development of the jaws and face. It may start from a common cold and persist

after its disappearance; or it may be due to a nasal obstruction, such as adenoids. It can easily be detected, and should have immediate attention from the rhinologist if there is obstruction. Removal of the obstruction alone is not sufficient to break the habit, and it should be followed up by the dentist with a long and continued series of prescribed vigorous breathing exercises, of which the patient should keep a personal record, to be checked up during his periodic visits. The typical appearance of advanced cases, which so often confront the orthodontist, is the "adenoid face" with a short upper lip, lack-lustre eyes, and a vacant expression. A serious disturbance of the balance of muscular forces results in contraction of the dental arches in almost every case.

There is a long list of the sucking or biting habits, such as of the lips, thumb, clothing, cheek, tongue, etc. All of them result in perversions of growth that complicate or produce malocclusion, such as protrusion of incisors, crossed bites of one or more teeth, and nonocclusion or open bite, which may occur in any locality, and in the advanced case may be so extreme as to allow occlusion on the points of only two opposing cusps. The elimination of these habits is indicated on the first appearance. Then there is disuse that may be induced by sore teeth which, if bilateral, removes the stimulus of exercise essential to growth, or if unilateral results in asymmetrical growth, which to the orthodontist is a very difficult problem. There are also habits of posture, which exert a very pronounced influence on the direction of growth. These are many and varied of which only two are mentioned: faulty sleeping habits with the face pillowed on the hand, or the habit of reading with the chin supported by the hand. The responsibility for the elimination of these and other habits logically rests upon the dentist, and their eradication would be a tremendous help to orthodontic procedure by minimizing or removing the causes of these disturbances of growth and development.

Neglect of the teeth in the past has been one of the most disturbing and most frequent of the complicating causes of malocclusion. There has been a distinct lack of coöperation from the dentist in the dental care of children. Many cases come to the orthodontist with mouths crippled by caries, abscesses, and loss of teeth, and

mothers still report the dentist's reaction in his comment that "they are only baby teeth" or "those cavities are too small to fill." An embarrassing situation arises with the orthodontist endeavoring to fulfill his obligation to the case without destroying the parents' confidence in the family dentist. With the advent of children's dentistry, which is taking a prominent part in our professional service, it is likely that this cause of malocclusion will be less frequent.

The time for eruption of the teeth has quite a wide range of variation above and below the average, without any adverse influence on the development of occlusion; but the law of succession of eruptions is much more important, because of the disturbance of occlusal relationships that follows variations in the successions. One of the provisions of a proper succession is that the mandibular teeth erupt before the maxillary mates, with the important exception that the upper premolars should erupt before the lower. This is one of the steps in the development of the curve of Spee, which is one of the essential attributes of adult occlusion. There are other disturbances of eruption that produce malocclusions of exaggerated type, and you are all familiar with the case, at the age of 12 or 13, which presents inadequate space for the eruption of the permanent bicuspids. Reading back those cases to the deciduous dentition: the condition that prevailed at that time doubtless included a lack of lateral development, with a crowding of the incisors, and in many cases the exfoliation of the deciduous central and lateral for the accommodation of the permanent centrals, followed by the exfoliation of the deciduous cuspids at the time of the eruption of the lateral. Such occurrences indicate the immediate necessity of gaining added width of the arches. One of the disasters that follows a premature loss of the deciduous molars is the consequent drift of the remaining teeth owing to the break in the neighborly contact of the arch, and eventually results in the displacement or impaction of one or more of the premolars. This situation in the developed case of malocclusion presents a technical problem that is particularly difficult to correct. It is easy for the dentist to prevent its occurrence by applying a space maintainer,

immediately after the loss of the deciduous tooth, that will prevent the drift of the remaining teeth and the subsequent impaction.

It would seem unnecessary to call attention to improper restorations that do not restore the normal contact of neighboring teeth or proper cusp and sulcus relationship of the occlusal surfaces. And yet it is not a rare occurrence to see violations of these established technical procedures. From the illustrations given, it is clear that a knowledge of the etiology of malocclusion is essential to the dentist. Further than this, there should be in his mind a fairly definite idea of what constitutes a "norm" in occlusion.

The orthodontic concept to-day asserts that the norm is individual, modified, and influenced by the peculiar hereditary and environmental forces pertaining to that individual, but with certain fundamental characteristics of relationships in the three dimensions of space within reasonable limits of variation. There must be in any norm a width of arch sufficient to accommodate the definite number of teeth in a characteristic human curvature. There should also be a fair degree of both lateral and antero-posterior symmetry, with a close registration of the upper and lower median lines. The antero-posterior relation of the mandible may not deviate very far from the evident cusp relationships that have been developed by ages of adaptation.

Particular emphasis is placed upon the fact that there are very appreciable differences in the arrangement of the deciduous dentition as compared with the adult, or the mixed dentition. It is essential, therefore, that the dentist should be thoroughly conversant with changes that take place in the occlusion of the individual, from the appearance of the first tooth in infancy to the complete development of the adult. During this period there occurs an intensive growth of all the surrounding and supporting structures in all three dimensions, with a commensurate change in occlusal relations. While this growth is continuous, there are periods of retardation and acceleration in any one direction, and zones in which it occurs. Compare the height and weight of the infant to the adult, and contrast the number and size of the deciduous with the permanent teeth, and a startling conception of the amount occurring in a few

years can be obtained. And yet efficient occlusion and harmony of relations of all the parts is maintained throughout.

The wide acceptance of the principles of preventive dentistry that has occurred in the last few years is a matter of greatest interest and concern to the orthodontist, because the practical application in the dental office of proper dental attention to pits and fissures holds the alluring possibility of eliminating many of the environmental, causative factors of malocclusion and their distressing sequelae, some of which have already been mentioned in this paper. But, entirely aside from the province of this paper, it seems opportune to call attention to the fact that even this desirable and necessary approach does not go back in preventive effort to the basic cause of the whole situation, which must lie in the field of heredity, or in constitutional or functional disorders beyond the field of dental endeavor that demand the preventive effort of medicine in the pre-natal and infancy periods of life.

The orthodontist eagerly promises every possible aid for dental prevention because of the evident and unquestioned advantage for the individual in the course of growth and development; and he very humbly asks for the coöperation of the dentist to modify and adjust his technical procedure to include measures in harmony with the requirements of that same growth and development essential to the assurance of efficiency in occlusion.

IV. DIET AS A FACTOR IN PREVENTIVE DENTISTRY

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We have experimental evidence that well-developed and normally nourished enamel is highly resistant to the agencies that cause its breaking down, presumably by the by-products of the Bacillus acidophilus. We also have abundant evidence that well developed and normally nourished dentin is practically immune to the invasion of the proteolytic organisms that are supposed to produce its disintegration. I wish to speak of the factors that render dentin susceptible to the invasion of these proteolytic organisms, and which predispose it to that type of caries that results in the familiar rapid disintegration.

The study of our clinic patients indicates that there are generally two factors associated with high susceptibility to decay. The first is the astonishing prevalence of secondary anemia. In testing the blood of children and adults we found that about 77 per cent of all who present themselves for dental treatment are suffering from secondary anemia. We also found that they are suffering from a persistent and long enduring calcium imbalance. The principle we are working on to combat these two conditions is fundamental, and that is, that nothing can be done in the restoration of tissue without a normally constituted blood stream. This brings up the problem of the treatment of secondary anemia, and consequently raises the question of the function of iron in metabolism. So far as I am aware, iron in metabolism has only three functions, namely, the development of hemoglobin; the development of the so-called chromatin bodies in the nuclei of cells, which are necessary to their normal chemical action; and to act, directly or indirectly, as a catalyst in the muscular tissue, where the chemical energy processes go on, and by which the life processes are kept moving.

Hemoglobin is made up of two constituent parts, hematin and globulin. We are powerless to control the development of globulin; it is, however a very simple matter to control the development of hematin, which is composed of two essential factors. The first is a closed-ring compound and has received the name pyrrole. The body cannot synthesize a closed-ring compound, but it can synthesize chemical compounds in carbon chains that are direct and in line. No individual can build normal blood who does not take into his body an adequate amount of pyrrole derivative compound. Hematin is composed of four pyrrole nuclei, which must come from the outside (contained in the food), and are bound together by one atom of ferric iron. That is the basis for the building of hematin. Presently I shall show you some slides[2] to demonstrate that you can feed an animal all the pyrrole and iron you please, and it will not build hematin. There is another and a more determining factor, and that is the presence, in the food or otherwise, of a very small amount of catalyst to effectively combine pyrrole molecules with

[2] The illustrations were not presented for publication.

ferric iron to build hematin—in this case, either copper or manganese. Therefore, an individual who is so dieting that he is not getting an adequate amount of pyrrole in his diet, or whose diet does not contain something that carries either copper or manganese, must inevitably develop secondary anemia. Nature makes a wise provision for just this sort of thing. There is no mother's milk which is adequate for the normal nutrition of her offspring unless that mother has dieted judiciously before the child is born. Nature stores in the body of the child elements which will not be in the mother's milk. When that condition has been adequate and well balanced, the child will not suffer from secondary anemia. If, on the other hand, the mother has been living during the pre-natal period very largely on cereal grains and dry toast, and things of that sort, she has not stocked the unborn child with the necessary catalytic agents and the result is that it will develop a secondary anemia.

I do not have any data regarding these catalytic agents in the human species, but I do have it for the lower animals. It has been found in a calf that is yet unborn (let us say when the two-thirds incubation period is past) that its liver contains 908 milligrams of copper. If it is allowed to come to full term, and then is killed ten days thereafter and its liver is analyzed, it is found to contain 400 milligrams. In other words, during the last period of gestation and the first ten days of life, this animal actually uses perhaps 50 per cent of the normal stock of copper, as a catalyst, to build its initial blood supply to the normal standard. If the liver of an ox, in his normal habitat and normal activity be analyzed, it will be found that it contains only about 50 milligrams. Why? He does not need to stock this, because the diet he is using supplies this catalyst every day. But if he does not stock copper, he will develop secondary anemia. Therefore, a normally nourished child that is to escape secondary anemia during the lactation period must be born with a stock of this catalyst, either copper or manganese. What is true of copper and manganese is true also of iron. It is an interesting fact that a child during the nursing period contains six times as much iron in its body as is put there by the milk it drinks. During the lactation period a child has three times as

much iron to its total body-weight as has an adult. When the child is weaned at the ninth or tenth month, it has practically increased its weight threefold. Therefore, when the child begins to eat solid foods, the percentage of iron in its body is the same as that of an adult. If the child at this stage of life be fed on a diet poor in pyrrole, poor in iron, and poor in catalysts, then that child will suffer from secondary anemia.

To illustrate the great problem of the composition of foods, which is involved in nutritional imbalance, I call your attention to the fact that every individual, from the third year of age until he is advanced in years, needs every day for his physiological purposes 15 milligrams of iron, the amount that is eliminated daily from the body as the result of catabolic processes. If he should depend entirely on milk, he would have to drink every day two gallons and a half. When we want to produce secondary anemia in rats, we feed them on milk only. The same thing will happen to a child unless he has been already stocked, pre-natally, with these necessary inorganic ingredients. If we want to limit his diet to meat, we must feed him every day 600 grams of meat. If we choose to derive the iron from eggs or wheat, we must feed him 600 grams of eggs or 400 grams of wheat. If we want to derive it from spinach, we must give him 600 grams of spinach. No child is going to eat 600 grams of eggs, 400 grams of wheat, or 600 grams of spinach.

The great nutritional problem is: how can the necessary amount of iron be supplied, in order to prevent secondary anemia? The second and more serious proposition is: how can pyrrole be supplied and what are its sources? There is only one constituent in milk, meat, wheat, and other cereals that can possibly supply this necessary derivative for the building of blood, and that is one of the so-called amino-acids, known as tryptophan, a very complex molecule. It contains pyrrole bound to another closed ring. When the body digests meat it has the great difficulty of breaking one ring from the other, so that the pyrrole will become available. The result is that an individual depending on this source for his pyrrole is likely to develop secondary anemia. We also observed, in the cases of late dentition—fifteen months overdue from the normal time of dentition—that in every case there was secondary anemia.

Where can pyrrole be found? You can get it from meat, but its abstraction is difficult. Pyrrole is supplied very abundantly in the working parts of green vegetables, to which Dr. Leonard has just alluded as important ingredients of a balanced diet. Of the plants that possess pyrrole in the most available and most concentrated form there is one which unfortunately we do not use on the table, and that is alfalfa. In order to combat this condition in our dental clinic, we have worked out a method of extracting pyrrole from the ordinary alfalfa leaf, which is very green, very thin—a high, working leaf. Then we put it either in tablet form or in a capsule, or work it up into honey for the very small children. We give the patient this medication at least twice a day in order to be certain that he will have an adequate amount of pyrrole in the diet. In addition to that, we give him iron, which he must have, and to the iron preparation we add a little of one of the catalytic agents—copper or manganese. The results of our experimental research show that on the average we can build 100,000 red blood cells per cmm. of blood every seventh day.

A man 72 years of age, who came recently to my office, was suffering from pernicious anemia, and had been for a number of years. He was taking liver extract, but he was gradually growing worse. On January 11 of this year we put this old man on the extract from the alfalfa plant, together with iron preparation, made up in a syrup with a little catalyst, manganese or copper. On January 27 the technician at the city hospital made a blood count, which showed that between January 11 and 27, the patient built 1,168,000 red blood-cells per cmm. His blood cells had returned to normal—5,000,000 cells per cmm. In addition to the numerical increase in the cells, their morphology was now normal, which was an important observation because in pernicious anemia the red blood-cells have a crenated character—they are disintegrating. There remains only one defect in this old gentleman's blood; and that is, the defense cells are not as high as they should be. We have never yet treated a case of secondary anemia in children that was not restored to normalcy within two or three weeks—at the most three. When we combat a case of dental caries, where the dentin is breaking down

very rapidly, that is the first thing to do. It costs about $1.75 to bring a child to normalcy from this standpoint.

Having thus established normalcy of the blood, we then attack the recalcification of dentin. I have had abundant clinics to be perfectly sure of the statement that you can take a dentin that is so soft that a probe will penetrate it, and in the course of two or three months you can make it almost as hard as the enamel. What is happening in the progressive decalcification of the dentin that makes it so open to the invasion of the proteolytic organisms? It all goes back to a persistent nutritional imbalance of calcium. I should like to submit just a few figures to show how easy that is to understand. Sherman, and other physiological chemists, in studying the calcium requirements of an individual, make the statement that on the average a child between the third and thirteenth year (you can extend that if you please and put it from the third to the seventeenth year) stores in his body every day 2 grains of lime. In addition to that, he must use every day for his physiological purposes 15 other grains of lime. In order, therefore, to get his normal structure and to maintain that structure in its integrity, he must take into his body every day 17 grains of calcium. If you are going to depend on milk to get the calcium it would be necessary to drink every day 1000 cc. In the outline for combating dental caries, we always suggest that the individual use every day one quart of milk. One reason is that it yields 15 grains of lime, but there is another reason. Sherman made the following interesting experiment. To children between the ages of 3 and 13 he gave 750 cc. of milk (which would not contain 15 grains of lime), and added just enough other food to make up 15 grains of lime, and estimated how much of the calcium was assimilated. To another group of children he gave 1000 cc. of milk, and cut down the diet so that the total of contained lime was 15 grains. In this experiment the children took daily the same amount of calcium into the body, but one group received 750 cc. of milk, the other received 1000 cc. of milk. It was found that the second group assimilated 70 per cent more lime that the first. Therefore, in treating a case of dental caries we always prescribe 1000 cc. of milk, not only for

the sake of the calcium in it, but also because of the high degree of calcium assimilation it induces in very young children.

Bone cannot be built unless there is an adequate amount of phosphorus. A child given calcium alone will not build bone if there is not an adequate amount of phosphorus in the diet. Calcium imbalance may develop a phase of rickets known as calcium rickets. By the same token, phosphorus rickets may be produced by a negative dietary balance of phosphorus. The result of extensive work on the part of various physiological chemists indicates that if you give a child between the age of 3 and 13, when he is building bone very rapidly, about 14 grains of phosphorus in the diet, he eliminates more phosphorus than he has received. In other words, he is in negative balance. But, if you give this individual about 22 grains of phosphorus in the diet, he will nourish his bones normally and will store a little phosphorus.

What are the possibilities when we feed our children? If we give them enough milk to supply all the calcium they need, say 1000 cc., that amount of milk is just 50 percent short in the required amount of phosphorus. Wheat is very rich in phosphorus and very poor in calcium. Therefore, if you combine milk with an abundance of wheat, you may strike the balance. Therefore, the great problem in building bone, and in maintaining the nutrition of the dentin, is to get the right balance of the calcium and the phosphorus requirements. If the body does not receive an adequate amount of phosphorus, it will use what it receives for building brain tissue or nuclei of the cells, which keep the life processes going, and let these hard tissues suffer. From the study of 3000 diets I found that these individuals got two-thirds of the required amounts of calcium and phosphorus to meet body needs. Therefore, we have just compounded, in order to meet this situation, a tablet that is made up of calcium and phosphorus in the ratio in which they are to be found in dentin. They are 5-grain tablets and we prescribe two of them every day, to give two-thirds of the requirements. We trust the diet for the other third.

The question has often been asked: "What will happen if you give me too much?" I shall answer that. The body fixes a norm, and that norm is determined by some process or some mechanism

that we know nothing about. We took seven adult men in good health, with normal occupations, and we gave them a diet we knew would supply all of the calcium their bodies needed per day; namely, 15 grains. In addition to that, we gave each one 90 grains of calcium daily, so that each received 105 grains of calcium every day. That experiment was continued for seven days. At the end, the blood of each was analyzed. Three lost 0.1 per cent of calcium; the other three gained 0.1 percent. When you struck the average, there was no change whatever. In other words, the body will use what the body needs, how we do not know, and the unnecessary part will simply be eliminated. If such a provision as that were not made in nature, of course, we would all have a nutritional imbalance at all times.

That brings up another question; namely, the conditions necessary for the assimilation of calcium. Dr. Leonard has pointed out one, namely, the mental state, which has a great deal to do with it. A man, for instance, who is very much worried over his business will have his teeth decay, in all human probability. The mother who is suffering from family worries will have calcium deficiency. The nervous equilibrium undoubtedly has much to do with it. Then there must be an activator present, and that activator can be either the ultraviolet lamp or direct sunlight, or cod liver oil, which contains an abundance of the activator known as vitamin D. At present, when we do not have strong sunlight, when the active part of it is cut out because of the sodium-calcium glass behind which we are sitting, when the foods that we are eating are very largely deprived of the activator vitamin D, all Nature, during the winter months particularly, is conspiring against the integrity of the hard tissues to prevent successful assimilation.

In conclusion, I wish to reiterate that we have taken dentin that was white and soft as cottage cheese, and at the end of five months we have made from it a perfectly normal yellow dentin that would yield the characteristic polish with the burr, that would give the phenol reaction, and that was pronounced to be perfectly normal dentin. We have had seventy-five cases in which the teeth were condemned, and at the end of five months these teeth were pronounced perfectly safe.

THE PENETRATIVE POWER OF THE BACTERICIDAL RAYS IN THE ULTRAVIOLET PART OF THE SPECTRUM, AS EMITTED BY CARBON C

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I. PENETRABILITY OF GLASSES

More important than the inherent resistance of organisms, as an obstacle to the practical application of ultraviolet radiation therapeutically, are the low penetrative power of the bactericidal range and the avidity with which the rays are absorbed by organic matter. The penetrability of various glasses by the germicidal rays emitted by Carbon C was therefore investigated. The same materials and the same technique were employed as in previous experiments.[1] The petri dishes however, after being prepared, were covered with various glasses, and then exposed to the light emitted by Carbon C.

The results in *Table 1* demonstrated the usefulness of fused quartz, as it is very readily penetrated by the short rays. Results of ultraviolet transmission tests at the Bureau of Standards (1), upon a number of special glasses and common window-glass areas, follow (*Table 2*).

Although, according to the figures compiled at the Bureau of Standards (1) and listed above, Corex apparently allows the penetration of 89 percent of long wave-lengths, the results of our experiments

[1] Vorster: *Journal of Dental Research*, 1929, ix, p. 519.

indicate that it allows only a small percentage of the lethal or short rays to pass. The other three glasses in our experiments were found to be more or less opaque to these short rays, the limit of penetration of ordinary glass being 340$\mu\mu$.

TABLE 1

Germicidal effects of the ultraviolet light given out by Carbon C, on Staphylococcus aureus after passing through various glasses

FILTER	THICKNESS	2 MINUTE EXPOSURE	4 MINUTE EXPOSURE	8 MINUTE EXPOSURE	20 MINUTE EXPOSURE
	mm.	Only 2 colonies present	Lethal	Lethal	Lethal
Quartz	2.5	Definite single colonies	Lethal	Lethal	Lethal
Corex	3.5	No effect	Slight inhibition	Definite check many single colonies	Lethal
Quartz-lite	2.0	" "	No effect	No effect	No effect
Corning	11.5	" "	" "	" "	" "
Petri dish lid	2.0	" "	" "	" "	" "

TABLE 2

Percentage transmission of ultraviolet light at 302 $\mu\mu$ (Å 3020)[1]

	THICKNESS	PERCENTAGE
	mm.	
Fused quartz	4.7	92
Corex	2.8	89
Quartz-lite	1.9	0.5
Window glass	3.3	0.0

[1] $\mu\mu$ = unit of measurement—0.000,001 mm. Å = Angstrom unit—0.000,000,1 mm.

II. EFFECTS OF SENSITISERS

It has been suggested that the penetrative power of the lethal rays could be increased by the use of various dyes, or fluorescent substances, as sensitisers. In 1897 Otto Raab (2) by accident discovered that the killing action of acridin hydrochloride on protozoa (paramecium) was increased by the action of light. Raab found that only fluorescent substances show this photodynamic action, and therefore concluded that fluorescence was responsible for it. Following this, von Tap-

peiner (3) made extensive investigations with various dyes, and from the results of his experiments drew these conclusions:

(1) Light emitted by the fluorescent body is in itself ineffective, and it is necessary that the sensitiser be in contact with the material on which it acts.

(2) The only light that is effective is light of wave-lengths corresponding to the absorption band of the sensitiser; and that sensitising reactions of dyes are specific, in general, although eosin seems to be a sensitiser for all cells and chemical substances.

Victor Henri (4), employing colloidal selenium, threw light on the subject. The solution was flourescent but, in the dark, it did not affect all the protozoa; under the ultramicroscope it was seen to be a suspension of very minute particles. A certain number of organisms took up these particles into vacuoles, where they aggregated into small masses. On exposure to light it was found that only those organisms which had taken up the colloid were affected, thus showing the necessity for close contact and indicating a photochemical reaction—although not of a product that could act independent of light. If this had not been the case, those organisms which had not taken up the colloid would have been affected by the product that diffused from others.

The explanation of the phenomena seems therefore to be similar to that for the photographic plate. The dye is absorbed on the surface of the organism and the effect is probably produced by an activation of oxygen, or by a product of the oxidation of the dye, since it requires the presence of oxygen for the phenomena to occur. It is thus suggested that light of wave-lengths longer than 300$\mu\mu$ can be made as effective, in its destruction of bacteria, as light of wave-lengths shorter than 300$\mu\mu$, by the presence of sensitisers, which affect the surface conditions of the organisms, so that their photo-electric threshold is shifted into the visible. Only that region of which the wave-length is absorbed by the fluorescent substance is effective; for example, the longer red and yellow rays for methylene blue, the green and blue for eosin, and the violet and ultra-violet for quinine.

Following these investigations various dyes were employed therapeutically in conjunction with actinic therapy. These optical sensitisers increased the amount of available light at the area of irradiation.

thus obtaining greater effect, and secured, if possible, greater penetration. The results obtained, however, have in the main not been very satisfactory.

III. PENETRABILITY OF DYES

The writer investigated the action of some of the dyes on germicidal effects produced by the rays emitted by the various carbons with reference to Staphylococcus aureus. This was done in the hope of

TABLE 3

Germicidal effects of the ultraviolet light given out by Carbon C, on Staphylococcus aureus after passing through solutions of dyes

FILTER	2 MINUTE EXPOSURE	4 MINUTE EXPOSURE	8 MINUTE EXPOSURE	20 MINUTE EXPOSURE
	Two colonies still present	Lethal	Lethal	Lethal
Cell	Few colonies	"	"	"
Distilled water	" "	"	"	"
Gentian violet	Just an indication of inhibition	More definite	Many single colonies	Few colonies still present
Eosin	Very faint indication of inhibition	Slight effect	More definite	Some colonies still present
Methylene blue	Faint indication of inhibition	Definite check	Many single colonies	Some colonies still present
Mercurochrome	No signs of inhibition	Faint indication of inhibition	Slightly better effect	More pronounced but many colonies

obtaining greater germicidal action. The first step was to investigate the penetration by the germicidal rays given out by Carbon C when passed through a solution of a dye. A cell enclosed by fused quartz, and filled with a solution of a dye, was interposed between the rays emitted by Carbon C and the contaminated surface of the petri dish. Controls were run. The same technique was employed as in previous experiments, exposures ranging from 2 to 20 minutes. Solutions were used in a strength of 1–100 except for mercurochrome, which was used in a 1–4000 solution. (*Table 3.*) When, however, a sheet

of quartz-lite glass 2 mm. thick was interposed between the solutions and the surface contamination, no effects whatever were produced even after half an hour's exposure.

From these results, the conclusion may be drawn that, although the solutions absorbed a considerable amount of the lethal light, they are evidently somewhat penetrable to these lethal rays, to which quartz-lite glass is opaque. This penetration was found in our experiments to be more marked with gentian violet and methylene blue than with eosin, and more pronounced for eosin than mercurochrome. In the latter case only slight inhibitory effects were noticed.

In the next experiments, a few drops from known concentrations of a dye were added, by means of a sterile graduated pipette, to 24-hour broth cultures, thus obtaining desired concentrations of the dyes in the broth cultures, without diluting the cultures to an appreciable extent. Petri dishes were then contaminated as before with four drops from these dyed cultures and exposed as before to the rays emitted by Carbon C for 2, 4, 8, and 15 minutes, as well as for 8 and 15 minutes to the other carbons, respectively. The solutions used consisted of eosin, methylene blue, and gentian violet, in concentrations of 1–10, 1–100, 1–1500, and 1–4500 in the broth; of mercurochrome, in concentrations of 1–1000, 1–4000, 1–6000, 1–12,000, 1–30,000. The results obtained were all negative; that is, the effects produced on the organisms, although pronounced, were less than in the controls, so that a 4-minute exposure with Carbon C was not found to be lethal, whereas with controls it was. Similar results were obtained with all the other carbons. In no instance was a lethal effect produced. Furthermore, the inhibitory effects in the presence of the dye were more pronounced the more the dye was diluted. When a sheet of quartz-lite glass 2 mm. thick was interposed between the contaminated dyed surface and the source of light, no inhibitory effects were produced on the organisms even after 30 minutes exposure. The results of these experiments, therefore, suggest the following:

The inhibitory effects produced on Staphylococcus aureus, when the rays, emitted by the various National Therapeutic Carbons, fall upon the organism and sensitiser combined, are not improved by the

presence of the sensitisers used in our experiments, when the concentrations of these sensitisers are as given above. On the other hand, the presence of these sensitisers seems to decrease the inhibitory effects produced on these organisms by the various carbons.

Such effects as were produced in the case of the organism and sensitiser combination are evidently due merely to a penetration of lethal rays, and not to the photodynamic action of the sensitisers. Clinicians are apparently mistaken in thinking that these dyes sensitise to the ultraviolet light. Our experiments suggest that these dyes do not improve the germicidal action of the rays given out by the National Therapeutic Carbons—in fact, their presence seems to be a hindrance to this action. The use of these dyes in actinic therapy seems, therefore, to be of no apparent value, when a carbon arc and the National Therapeutic Carbons are employed.

The non-irradiated field of the contaminations containing a 1–10 solution of eosin, and to a certain extent in those containing a 1–10 solution of methylene blue, seemed to be affected as the colonies were much larger than usual. A similar increase in the size of the colonies, within the irradiated areas, was noticed in our earlier experiments with the different carbons. It was quite evident that there was a definite increase in the size of the colonies at a certain exposure. If exposure was prolonged a diminution took place in the size of these colonies, this diminution progressing as the time of exposure was increased; and, in the case of Carbon C, was followed by destruction. The time required for this to occur varied slightly with the different carbons, but was more or less limited to between 6 and 8 minutes exposure.

This phenomenon was also noticed when contaminations containing mercurochrome 1–4000 were used. In solutions of 1–1000 no growth had taken place after 24 hours, while solutions of 1–6000 showed a good even growth. Bovie (5), experimenting with paramecium, stated that with a sublethal dose inhibition is followed by an acceleration of cell division which, for a short exposure, may give an increase in the number of radiated individuals over the controls.

This seems to be the case in all our experiments with the various carbons, similar action having been noticed in the non-irradiated field when certain concentrations of the dyes were employed.

IV. PENETRABILITY OF SALIVA AND SKIN

Saliva. Investigations of the penetration by the ultraviolet rays through skin have been undertaken by many. The penetrability of saliva, however, has been neglected; although, in the application of actinic therapy in dental disturbances, the penetrability of saliva is a factor that cannot be disregarded. An investigation of the penetrability of saliva was, therefore, undertaken, employing the same technique as that in the experiments with solutions of the dyes.

A cell containing saliva was interposed between the rays and the contaminated surface of the agar in a petri dish. The cell and the petri dish were so arranged as to be both perpendicular to the base of a supporting stand as well as parallel to each other. The lamp was adjusted so that the central ray would strike the surface of the cell and the petri dish at an angle of 90 degrees, the rays passing through the cell at a point below the location of the air bubbles in the saliva. This arrangement eliminated the possibility of shadows being cast by the air bubbles in the saliva and thus interfering with the penetration by the rays. Exposures ranged from four to thirty minutes.

The results obtained were all negative; that is, no signs of inhibition were present even after exposures lasting for 30 minutes. Controls were run, in which the cell filled with sterile distilled water was interposed instead of saliva. These controls showed total destruction of the organisms after four minutes exposure. The above results suggest, therefore, that saliva is evidently to a great extent opaque to the lethal rays. This great absorption of the lethal rays by the saliva may be due to its organic constituents, as it is found that the lethal rays are very readily absorbed by organic matter.

Results of our investigation suggest that an analysis of the rays that penetrate saliva ought to be undertaken. It is thus evident, from the results obtained, that if actinic therapy is to be applied to oral lesions, these areas should be kept as free from saliva as possible in order to obtain a sufficient germicidal action.

Skin. Among others, Hasselt, Henri and Glitscher (6), from the results obtained in their experiments, agree that ultraviolet rays penetrate skin only to the depth of 0.1 mm. Hasselbalch (7), determining the percentage of radiations of various wave-lengths penetrating 0.1 mm. of skin, obtained the data in *Table 4.*

According to these findings, it seems that the penetration by the bactericidal range of ultraviolet light is so meager that the therapeutic application of its germicidal power is of little value. Macht and Anderson (7), however, do not agree with these conclusions. They found, while experimenting with rabbits, that rays of wave-lengths 302.5$\mu\mu$, and even some of 280.3$\mu\mu$, not only penetrated living skin to a depth of 1 mm., but in parts actually passed through it and thus at least affected the surface capillaries. Their explanation of the findings of other investigators is that most of them were obtained with dead skin. The results obtained in these experiments seem to indicate that there is a decrease in the penetration of the rays through dead skin as compared with living skin.

TABLE 4

Percentage of ultraviolet light of various wave-lengths that penetrated 0.1 mm. of skin

WAVE-LENGTHS	PERCENTAGE PENETRATING 0.1 MM. OF SKIN
$\mu\mu$	*per cent*
302	8
297	2
287	0.01

Following along lines similar to that of Macht and Anderson, an investigation was made of the effects of the lethal rays given out by Carbon C on Staphylococcus aureus after passing through skin, with the object of determining whether any penetration of the rays through 0.1 mm. of skin is of sufficient concentration to produce germicidal effects on Staphylococcus aureus. In these experiments we employed rabbits, which were alive during the irradiation. The experiments were conducted as follows:

Anesthesia was produced with $\frac{3}{8}$ grain of morphia, which was injected subcutaneously. While waiting for the narcotic to take effect, petri dishes were prepared and contaminated as outlined in our previous experiments.[2] These were then placed in an incubator at 37°C. while the rabbit was prepared. The hair of the rabbit on the right side was now removed with a razor and a flap of skin turned back. The lamp was next adjusted, using Carbon C, and was so arranged that

[2] Vorster: *Journal of Dental Research*, 1929, ix, p. 519.

the central ray would strike the culture medium of the petri dish at right angles, the distance from the arc being 315 mm. Following this, the rabbit was placed next to a stand, a petri dish removed from the incubator was placed in position, and the flap pulled over and held in position with hemostats. The flap of skin was about 5 mm. from the surface contamination. Exposures for 4, 8, and 30 minutes, respectively, were next made through this flap of skin at two different points. The thickness of the flap of skin at these two points measured 0.88 and 1 mm. respectively.

The results obtained following these exposures were all negative. There were no signs of inhibition on examination of the petri dishes the following day, good even growth being present in all the dishes.

In another experiment, 2 cc. of a 2 per cent solution of novocain and 0.002 per cent suprarenin were injected into the flap of skin and the tests carried out as outlined above. In a third experiment, the ear of the rabbit was shaved and an exposure of 30 minutes made through the ear.

The results obtained in these two experiments were similar to those of the first experiment with the skin of the rabbit. No signs of inhibition were to be seen the next day. Controls as usual showed total destruction of the organisms following a 4-minute exposure.

The skin was next removed and placed within sterile gauze. The following day experiments similar to those outlined above were conducted, using this dead flap of skin as a screen over the petri dishes. This flap was next preserved in formalin and used as a screen over the petri dishes in conducting experiments with it a week later.

The results of both these experiments were the same; namely, no inhibitory effects on the growth of the organism were to be seen, even after 30 minutes exposure.

A study of the results obtained in our experiments with the skin of a rabbit suggests, therefore, (1) that, if there is a penetration of ultraviolet light through 0.1 mm. of skin, this penetration is not of sufficient concentration to produce inhibitory effects on the growth of Staphylococcus aureus, when a carbon-arc lamp with the National Therapeutic Carbon C as electrodes is employed; and (2) that the injection of a 2 per cent novocain-suprarenin solution into the skin does not seem to improve the penetration of the lethal rays as given out by Carbon C.

We find, in studying the literature, that many investigators—among them Finsen (8)—are of the opinion that the penetration of the skin by ultraviolet light is dependent on the blood content of the dermis. Blood is found to be opaque to ultraviolet rays. Bovie (5), while investigating the action of ultraviolet light on the complementing power of serum, found that hemoglobin was responsible for the avidity with which the blood absorbs the ultraviolet rays. Furthermore, investigators agree that ultraviolet light of wave-lengths 300$\mu\mu$, and greater, penetrate to the basal cells of the epidermis with a resultant formation of the pigment melanin. Melanin is assumed to be the end product of the oxidation of tyrosin, or of one of the related cyclic compounds. It is suggested that this is probably brought about by the agency of an oxidising ferment such as tyrosinase. This presence of pigment is thought to provide a maximum absorption of radiant energy in the basal cells of the epidermis, thereby increasing the amount of available light at that point.

The results obtained in our investigation of the penetrative power of the lethal rays, together with the fact that rays of 300$\mu\mu$ and longer are found to penetrate to the deep epidermic cells, seem to suggest that most of the effects produced by the application of ultraviolet light therapeutically are due to rays of wave-lengths greater than 300$\mu\mu$ and not to the lethal or short rays. While these lethal or short rays would produce a decided germicidal action superficially, their penetrative ability seems to be such as to discourage their useful application in deeper areas. The therapeutic application of the longer rays, that is, rays of 300$\mu\mu$ wave-lengths and longer, is found to increase the red-cell count and the percentage of hemoglobin, as well as the quantity of calcium and phosphorus. These factors, together with the hyperemia and the germicidal action of the lethal rays superficially, are the important factors to consider in actinic therapy.

Although the local application of the lethal rays in dentistry seems, therefore, to be discouraged, owing to their limited power of penetration, general irradiation is of great value. Classens (9) states that pigmentation of the skin increases the resistance of the body to exposure, to difference in temperature, and to infections generally. Pigmented skin is less amenable to disease of the skin, cutaneous disease being rare among Kaffirs (African aboriginees).

V. CONCLUSIONS

1. Quartz glass is very readily penetrated by the lethal rays of ultraviolet light. Corex glass allows the penetration by but a small percentage of these rays. Quartz-lite, Corning, and ordinary glass are practically opaque to these rays.

2. Gentian violet, methylene blue, and eosin, in concentrations of 1 to 100 or less, and mercurochrome, 1 to 4000 or less, used as sensitisers, do not enhance the germicidal action of the rays of Carbon C when applied to Staphylococcus aureus, but tend to decrease this action.

3. Gentian violet, methylene blue, and eosin, in concentrations as high as 1 to 100, and mercurochrome, in concentrations as high as 1 to 4000, allow sufficient penetration of the lethal rays of Carbon C to inhibit to some extent the growth of Staphylococcus aureus.

4. Saliva is opaque to the lethal rays.

5. The germicidal rays of Carbon C do not penetrate a layer of dead, or living, rabbit skin (0.88 mm. thick) sufficiently to inhibit the growth of Staphylococcus aureus.

6. The germicidal rays of Carbon C do not penetrate the living tissues of a rabbit's ear sufficiently to inhibit the growth of Staphylococcus.

7. The injection of a 2 per cent novocain-suprarenin solution does not perceptibly assist the penetration of the skin by the lethal rays.

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BIOCHEMICAL STUDIES OF STREPTOCOCCI

V. Availability of Inorganic Substances[1]

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I. INTRODUCTION

This paper treats only a few outstanding results. The inorganic nitrogen compounds are not considered here since they have been dealt with in a previous paper.[1]

II. METHODS AND RESULTS

1. *Viability of streptococci in media containing only inorganic substances.* Eighty such media have been studied. Details regarding their respective compositions and value are given below. For convenience and clarity they are arbitrarily classified into groups A, B, C, D, on the basis of comparative gradations in the amount and duration of growth.

Group A

Media that showed growth in more than two subcultures, or abundant growth (4+) in at least two subcultures

Medium 234 (27): NH_4Cl, 0.01%; $Ca_3(PO_4)_2$, 1.0%; $MgCl_2$, 0.01%; KH_2PO_4, 0.3%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%; 0.05 cc. NaOH(2.123M) per 5 cc.

[1] The previous papers in this series were published in the *Journal of Dental Research*, 1929, ix: (1) Krasnow and Gies, p. 29; (2) Krasnow and Rosenberg, p. 123, (3) p. 321; (4) Krasnow, Harrow and Reiner, p. 531.

[2] A preliminary report was published in the *Journal of Dental Research*, 1928, viii, p. 458.

Growth: 4+, 4+, 4+, 3+, 6c, in the first five subcultures of the first transplant; +, +, 4c, 2c, in the first four subcultures of the second transplant.

Medium 167 (5): $CaCO_3$, 2.0%; KNO_3, 0.01%; K_2HPO_4, 0.02%; Na_2CO_3, 0.02%; sulphur, 10.0%. *Growth:* 4+, 4+, 4+, 3+, 3c, 3+, in the six subcultures of the first transplant; +, 5c, in the first two subcultures of the second transplant.

Medium 168 (5): $CaCO_3$, 2.0%; $MgCl_2$, 0.01%; KNO_3, 0.05%; K_2HPO_4, 0.02%; Na_2CO_3, 0.02%; sulphur, 10.0%. *Growth:* 4+, 4+, 3+, +, +, +, in the six subcultures of the first transplant; +, 3c, in the first two subcultures of the second transplant.

Medium 86 (26): $(NH_4)_2SO_4$, 0.02%; $Ca_3(PO_4)_2$, 0.25%; $MgSO_4 \cdot 7H_2O$, 0.5%; KH_2PO_4, 0.1%; sulphur, 1.0%; 0.028 cc. NaOH(2.123M) per 5 cc. *Growth:* 4+, 3+, 2+, 2+, in the first four subcultures of the first transplant; 1c, in the first subculture of the second transplant.

Medium 627 (9): $Fe_2(SO_4)_3 \cdot 9H_2O$, trace; $MgNH_4PO_4 \cdot 6H_2O$, 0.2%; $MgCO_3$, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.1%; NaCl, 0.2%; 0.005 cc. NaOH (2.123M) per 5 cc. *Growth:* 3+, 3+, +, +, +, in the first five subcultures of the first transplant; 2c, in the first subculture of the second transplant.

Medium 65 (25): NH_4Cl, 0.01%; $CaCl_2$, 0.025%; KH_2PO_4, 0.3%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%; 0.048 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, 4+, in the first two subcultures of the first transplant; 4c, in the first subculture of the second transplant.

Medium 232 (29)[3]: $(NH_4)_2SO_4$, 0.2%; $Ca_3(PO_4)_2$, 0.05%; $FeSO_4 \cdot 7H_2O$, 0.001%; $MgSO_4 \cdot 7H_2O$, 0.05%; KH_2PO_4, 0.5% sulphur, 1.0%; 0.08 cc. NaOH (2.123M) per 5 cc. *Growth:* 3+, +, +, in the first three subcultures of the first transplant.

Medium 15 (22): $(NH_4)_2CO_3 \cdot H_2O$, 0.5%; $CaCl_2$, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.5%; K_2HPO_4, 0.5%. *Growth:* 2+, 2+, 2+, in the first three subcultures of the first transplant; +, +, in the first two subcultures of the second transplant.

Medium 15u (O)[4]: $(NH_4)_2CO_3 \cdot H_2O$, 0.5%; $CaCl_2$, 0.0002%; $Ca_3(PO_4)_2$, 0.0024%; $MgSO_4 \cdot 7H_2O$, 0.5%; K_2HPO_4, 0.5%; 0.065 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+, 2+, +, in the first three subcultures of the first transplant; 1c, in the first subculture of the second transplant.

[3] 0.05% $Ca_3(PO_4)_2$ instead of 0.5%, the amount used by Waksman and Joffe.

[4] Reference "O" here, and in subsequent media, indicates that the medium has been devised by the present authors.

Group B

Media that showed fair (2+) or good (above 2+) growth in at least one subculture

Medium 151 (27): $(NH_4)_2SO_4$, 0.02%; $CaCl_2$, 0.025%; $FeSO_4 \cdot 7H_2O$, trace; $MgSO_4 \cdot 7H_2O$, 0.05%; KH_2PO_4, 0.3%; sulphur, 1.0%; 0.063 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, 2+, in the first two subcultures of the first transplant.

Medium 50 (29): NH_4Cl, 0.01%; $CaCl_2$, 0.025%; $MgCl_2$, 0.01%; KH_2PO_4, 0.3%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%; 0.046 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, +, in the first two subcultures of the first transplant; 4c, in the first subculture of the second transplant.

Medium 80 (29): $(NH_4)_2SO_4$, 0.2%; $Ca_3(PO_4)_2$, 0.75%; $FeSO_4 \cdot 7H_2O$, trace; $MgSO_4 \cdot 7H_2O$, 0.05%; KH_2PO_4, 0.5%; sulphur, 1.0%; 0.091 cc. NaOH (2.123M) per 5 cc. *Growth:* 3+, +, in the first two subcultures of the first transplant; 3c, in the first subculture of the second transplant.

Medium 85 (28): $(NH_4)_2SO_4$, 0.2%; $Ca_3(PO_4)_2$, 1.0%; $FeSO_4 \cdot 7H_2O$, 0.001%; KCl, 0.05%; K_2HPO_4, 0.1%; sulphur, 1.0%; 0.009 cc. NaoH (2.123M) per 5 cc. *Growth:* 3+, +, in the first two subcultures of the first transplant.

Medium 81 (26): $(NH_4)_2SO_4$, 0.02%; $CaCl_2$, 0.025%; $MgSO_4 \cdot 7H_2O$, 0.05%; KH_2PO_4, 0.3%; sulphur, 1.0%; 0.055 cc. NaOH (2.123M) per 5 cc. *Growth:* 3+, 2+, in the first two subcultures of the first transplant.

Medium 628 (9): $Fe_2(SO_4)_3 \cdot 9H_2O$, trace; $MgCO_3$, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.05%; KCl, 0.2%; $NaNH_4HPO_4 \cdot 4H_2O$, 0.34%; 0.005 cc. NaOH (2.123M) per 5cc. Growth; 3+, 1c, in the first two subcultures of the first transplant.

Medium 15b (0): $(NH_4)_2CO_3 \cdot H_2O$, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.5%; K_2HPO_4, 0.5%; $CaCl_2$, 0.0025%; NH_4NO_3, 1.5%; 0.05 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+ in the first subculture of the first transplant.

Medium 646 (25)[5]: $(NH_4)_2SO_4$, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.05%; $FeSO_4 \cdot 7H_2O$, 0.001%; $Ca_3(PO_4)_2$, 0.25%; KH_2PO_4, 1.089%; sulphur, 1.0%; 0.212 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+ in the first subculture of the first transplant.

Group C

Media that showed poor growth (+ or several colonies) in one subculture

Medium 30 (19): $(NH_4)_2HPO_4$, 0.2%; $MgSO_4 \cdot 7H_2O$, 0.2%; K_2HPO_4, 0.4%; 0.05 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.003 cc. NaOH (2.123M) per 1 cc. after sterilization. *Growth:* 2c in the first subculture of the first transplant.

[5] We omitted 2.0% M/3 H_3PO_4, used by Waksman.

Medium 8 (22): $(NH_4)_2SO_4$, 0.1%; $FeSO_4 \cdot 7H_2O$, 0.04%; $MgSO_4 \cdot 7H_2O$, 0.05%; NaCl, 0.2%; KH_2PO_4, 0.1%; $4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$, 0.5%. *Growth:* 3c in the first subculture of the first transplant.

Medium 15e (0): $(NH_4)_2CO_3 \cdot H_2O$, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.5%; K_2HPO_4, 0.5%; $CaCl_2$, 0.0025%; KNO_3, 0.1%; 0.07 cc. NaOH (2.123M) per 5 cc. *Growth:* + in the first subculture of the first transplant.

Medium 7 (22): $(NH_4)_2SO_4$, 0.1%; $FeSO_4 \cdot 7H_2O$, 0.04%; $MgCO_3$, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.05%; NaCl, 0.2%; K_2HPO_4, 0.1%. *Growth:* + in the first subculture of the first transplant.

Medium 535 (30): $(NH_4)_2SO_4$, 0.2%; $MgSO_4 \cdot 7H_2O$, 0.05%, K_2PO_4, 0.1%; Na_2CO_3, 0.5%; $CaCl_2$, trace. *Growth:* + in the first subculture of the first transplant.

Medium 536 (30)[6]: $(NH_4)_2SO_4$, 0.4%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2PO_4, 0.1%; Na_2CO_3, 0.6%; $CaCl_2$ trace. *Growth:* + in the first subculture of the first transplant.

Medium 136 (23): $MgCl_2$, 0.25%; KNO_3, 0.1%; NaCl, 3.0%; $Na_2HPO_4 \cdot 12H_2O$, 0.05%; $Na_2S_2O_3 \cdot 5H_2O$, 0.15%. *Growth:* + in the first subculture of the first transplant.

Medium 82 (29): $(NH_4)_2SO_4$, 0.2%; $CaCl_2$, 0.025%; $FeSO_4 \cdot 7H_2O$, 0.001%; $MgSO_4 \cdot 7H_2O$, 0.05%; KH_2PO_4, 0.5%; sulphur, 1.0%; 0.095 cc. NaOH (2.123M) per 5 cc. *Growth:* + in the first subculture of the first transplant.

Medium 83 (28): $(NH_4)_2SO_4$, 0.2%; $Ca_3(PO_4)_2$, 0.25%; $FeSO_4 \cdot 7H_2O$, 0.001%; $MgSO_4 \cdot 7H_2O$, 0.05%; KCl, 0.05%; K_2HPO_4, 0.1%; sulphur, 1.0%; 0.05 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.002 cc. NaOH (2.123M) per 1 cc. after sterilization. *Growth:* + in the first subculture of the first transplant.

Medium 433 (4): $NH_4NaHPO_4 \cdot 4H_2O$, 0.2%; KCl, 0.05%; 0.01 cc. NaOH (2.123M) per 5 cc. *Growth:* + 1c, in the first and third subcultures of the first transplant.

Group D

Media that showed no growth

Medium 49 (2): NH_4Cl, 0.5%; $CaCO_3$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.1%; KH_2PO_4, 0.1%; 0.023 cc. NaOH (2.123M) per 5 cc.

Medium 49a (2)[7]: NH_4Cl, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.1%; KH_2PO_4, 0.1%; 0.03 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.004 cc. NaOH (2.123M) per 1 cc. after sterilization.

[6] This is an adaptation of Nathansohn's medium.

[7] This is an adaptation of Bainbridge's medium, which contains 0.1% $CaCO_3$.

Medium 77 (3): $CaCl_2$, trace; K_2PO_4, trace; NaCl, 0.5%; Na_2SO_4, 0.1%; 0.002 cc. NaOH (2.123M) per 5 cc.

Medium 166 (4)[8]: NH_4Cl, 0.01%; $MgCl_2$, 0.01%; K_2HPO_4, 0.02%; $NaHCO_3$, 0.01%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%.

Medium 169 (5): K_2HPO_4, 0.01%; $NaHCO_3$, 0.02%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%.

Medium 582 (10): NH_4Cl, 1.0%; $CaCl_2$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.2%; K_2HPO_4, 0.1%; NaCl, 0.5%; 0.02 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.002 cc. NaOH (2.123M) per 1 cc. after sterilization.

Medium 584 (10): $(NH_4)_2HPO_4$, 1.0%; $CaCl_2$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.2%; K_2HPO_4, 0.1%; NaCl, 0.5%.

Medium 586 (10): $(NH_4)_2CO_3 \cdot H_2O$, 1.0%; $CaCl_2$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.2%; K_2HPO_4, 0.1%; NaCl, 0.5%.

Medium 588 (10): KNO_3, 1.0%; $CaCl_2$, 0.01%; $MgSO_4 \cdot 7H_2O$, 0.2%; K_2HPO_4, 0.1%; NaCl, 0.5%; 0.01 cc. NaOH (2.123M) per 5 cc.

Medium 54 (12): $(NH_4)_2 SO_4$, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.01%; K_2PO_4, 0.075%.

Medium 117 (14): $(NH_4)_2SO_4$, 0.1%; $FeCl_3 \cdot 6H_2O$, 0.0005%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.1%; NaCl, 0.1%; 0.005 cc. NaOH (2.123M) per 5 cc.

Medium 118 (14): $NaNO_3$, 0.1%; $FeCl_3 \cdot 6H_2O$, 0.0005%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.1%; NaCl, 0.1%.

Medium 566 (15): $(NH_4)_2SO_4$, 0.017%; $CaCl_2$, 0.0055%; $MgSO_4 \cdot 7H_2O$, 0.016%; K_2HPO_4, 0.1%.

Medium 567 (15): $CaCl_2$, 0.01%; $MgSO_4 \cdot 7H_2O$, 0.02%; K_2HPO_4, 0.1%.

Medium 506 (18): $(NH_4)_2SO_4$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.5%; K_2PO_4, 0.1%; NaCl, trace.

Medium 507 (18): $(NH_4)_2HPO_4$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2PO_4, 0.1%; NaCl, trace.

Medium 539 (17): $(NH_4)_2SO_4$, 0.2%; $FeSO_4 \cdot 7H_2O$, 0.04%; $MgCO_3$, 1.0%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2PO_4, 0.1%; NaCl, 0.2%.

Medium 540 (17): $(NH_4)_2SO_4$, 0.3%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2PO_4, 0.1%.

Medium 541 (17): $(NH_4)_2SO_4$, 0.3%; $FeSO_4 \cdot 7H_2O$, 2.0%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2PO_4, 0.1%; 0.05 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.005 cc. NaOH (2.123M) per 1 cc. after sterilization.

Medium 542 (17): $FeSO_4 \cdot 7H_2O$, 0.4%; $MgSO_4 \cdot 7H_2O$, 0.3%; K_2PO_4, 0.5%; Na_2CO_3, 1.0%; NaCl, 0.5%; $NaNO_3$, 1.0%.

[8] This is an adaptation of Beijerinck's medium.

Medium 533 (33): K_2PO_4, 0.05%; Na_2CO_3, 0.1%; $NaNO_2$, 0.2%.

Medium 534 (33): $MgSO_4 \cdot 7H_2O$, 0.03%; K_2PO_4, 0.05%; Na_2CO_3, 0.05%; $NaNO_2$, 0.1%; NaCl, 0.05%.

Medium 537 (31): $(NH_4)_2SO_4$, 2.0%; $MgCO_3$, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2PO_4, 0.1%; $CaCl_2$, trace.

Medium 538 (30): $(NH_4)_2SO_4$, 0.1%; $MgCO_3$, 0.5%; K_2PO_4, 0.1%.

Medium 543 (32): K_2PO_4, trace; Na_2CO_3, 0.1%; $NaNO_2$, 0.2%.

Medium 79 (20)[9]: $CaCl_2$, 1.0%; NaCl, 0.5%; Na_2SO_4, 0.2%; KH_2PO_4, 0.1%.

Medium 79a (20): NaCl, 0.5%; $CaCl_2$, 0.1%; Na_2SO_4, 0.2%; KH_2PO_4, 0.1%.

Medium 137 (23): NH_4Cl, 0.1%; $MgCl_2$, 0.01%; $NaHCO_3$, 0.1%; $Na_2HPO_4 \cdot 12H_2O$, 0.02%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%.

Medium 139 (23): $MgCl_2$, 0.01%; KNO_3, 0.1%; $NaHCO_3$, 0.1%; $Na_2HPO_4 \cdot 12H_2O$, 0.02%; $Na_2S_2O_3 \cdot 5H_2O$, 0.2%.

Medium 140 (23)[10]: NH_4Cl, 0.1%; $MgCl_2$, 0.01%; $NaHCO_3$, 0.1%; $Na_2HPO_4 \cdot 12H_2O$, 0.02%; $Na_2S_2O_3 \cdot 5H_2O$, 0.2%.

Medium 464 (24): K_2HPO_4, 0.02%.

Medium 150 (27)[11]: NH_4Cl, 0.01%; $MgCl_2$, 0.01%; $NaHCO_3$, 0.1%; $Na_2S_2O_3 \cdot 5H_2O$, 0.5%; K_2HPO_4, 0.02%.

Medium 67 (34): $CaCl_2$, 0.5%; $MgCl_2$, 0.1%; KCl, 0.5%; NaCl, 0.5%.

Medium 670 (11): $MgSO_4 \cdot 7H_2O$, 0.1%; K_2PO_4, 0.05%; $Na_3PO_4 \cdot 12H_2O$, 0.1%.

Medium 626 (9): $(NH_4)_2SO_4$, 0.1%; $Fe_2(SO_4)_3 \cdot 9H_2O$, trace; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.1%; NaCl, 0.2%; 0.016 cc. NaOH (2.123M) per 5 cc.

Medium 629 (9): $Fe_2(SO_4)_3 \cdot 9H_2O$, trace; $MgSO_4 \cdot 7H_2O$, 0.03%; K_2HPO_4, 0.05%; Na_2CO_3, 0.1%; $NaNO_3$, 0.1%.

Medium 686 (16): $MgCl_2$, 0.25%; KNO_3, 0.1%; NaCl, 3.0%; $Na_2HPO_4 \cdot 12H_2O$, 0.05%; $Na_2S_2O_3 \cdot 5H_2O$, 0.2%; 0.032 cc. NaOH (2.123M) per 5 cc.

Medium 15a^2 (0): Basic mixture—$(NH_4)_2CO_3 \cdot H_2O$, 0.5%; $MgSO_4 \cdot 7H_2O$, 0.5%; K_2HPO_4, 0.5%—plus 0.075 cc. NaOH (2.123M) per 5 cc.

Medium 15a (0): Basic mixture, plus $CaCl_2$, 0.0025%; 0.075 cc. NaOH (2.123M) per 5 cc.

Medium 15a^2 (0): Basic mixture, plus $CaCl_2$, 0.0005%; 0.073 cc. NaOH (2.123M) per 5 cc.

[9] This is an adaptation of the medium devised by Sperry and Rettger, which contains 0.1% $CaCl_2$.

[10] This is an adaptation of Trautwein's medium, which contains 0.01% NH_4Cl.

[11] This is an adaptation of Waksman's medium, which contains 0.025% $CaCl_2$.

Medium 15b[2] (0): Basic mixture, plus $CaCl_2$, 0.0005%; NH_4NO_3, 1.5%; 0.065% NaOH (2.123M) per 5 cc.

Medium 15b[3] (0): Basic mixture, plus NH_4NO_3, 1.5%; 0.06 cc. NaOH (2.123M) per 5 cc.

Medium 15c (0): Basic mixture, plus $CaCl_2$, 0.0025%; KH_2PO_4, 1.0%; 0.055 cc. NaOH (2.123M) per 5 cc.

Medium 15c[2] (0): Basic mixture, plus $CaCl_2$, 0.0005%; KH_2PO_4, 1.0%; 0.065 cc. NaOH (2.123M) per 5 cc.

Medium 15c[3] (0): Basic mixture, KH_2PO_4, 1.0%; 0.045 cc. NaOH (2.123M) per 5 cc.

Medium 15f (0): Basic mixture, plus $CaCl_2$, 0.0025%; NaCl, 0.1%; 0.06 cc. NaOH (2.123M) per 5 cc.

Medium 15h (0): Basic mixture, plus $CaCl_2$, 0.0025%; Na_2SO_4, 2.5%; 0.03 cc. NaOH (2.123M) per 5 cc.

Medium 15j (0): Basic mixture, plus $CaCl_2$, 0.0025%; KCl, 0.05%; 0.03 cc. NaOH (2.123M) per 5 cc.

Medium 15k (0): Basic mixture, plus $CaCl_2$, 0.0025%; $FeSO_4 \cdot 7H_2O$, 0.001%; 0.03 cc. NaOH (2.123M) per 5 cc.

Medium 15l (0): Basic mixture, plus $CaCl_2$, 0.0025%; $NaNO_3$, 0.2%; 0.035 cc. NaOH (2.123M) per 5 cc.

Medium 15q (0): Basic mixture, plus $CaCl_2$, 0.0025%; M/2 H_2SO_4, 1.0%; 0.05 cc. NaOH (2.123M) per 5 cc.

Medium 15r (0): Basic mixture, plus $CaCl_2$, 0.0025%; M/3 H_3PO_4, 2.0%; 0.01 cc. NaOH (2.123M) per 5 cc.

Medium 15a[2]a (0): $(NH_4)_2CO_3 \cdot H_2O$, 0.5%; $CaCl_2$, 0.0005%; $MgSO_4 \cdot 7H_2O$, 0.25%; K_2HPO_4, 0.5%; 0.08 cc. NaOH (2.123M) per 5 cc.

The foregoing data show that a surprising number of the media were capable of sustaining the organism for different periods and at various planes of metabolism. A comprehensive view of the situation may be obtained from the data in *Table 1*, which show that (1) 33 per cent of the media containing only inorganic substances supported life to some extent,

(2) Eleven per cent of the media were comparatively good, 10 per cent fair, and 12 per cent poor,

(3) Eleven of the media (14 per cent) contained elementary S. This substance, then, was a constituent of 41 per cent of the media permitting growth—a large proportion,

(4) The media containing $Na_2S_2O_3$ are about equally divided

between groups A + B + C and D, four in the former and five in the latter, and

(5) Twelve media (15 per cent), containing neither S nor $Na_2S_2O_3$, gave positive results for growth. They constitute 41 per cent of the total number in groups A + B + C.

The frequency of positive results for media containing S is about equal to that for media from which S or $Na_2S_2O_3$ was absent. However, all the media containing S gave growth; 9, or 82 per cent, are in the best groups (A and B), while only 2, or 18 per cent, are in the poor group, C, and none in the negative group, D. On the contrary only 12, or 20 per cent, of the media containing neither S nor $Na_2S_2O_3$ permitted growth. The percentage rapidly increases with *decrease in*

TABLE 1

Summary of the findings for the four groups (A—D)

GROUP DESIGNATION	NUMBER OF MEDIA IN EACH GROUP	PER CENT OF TOTAL	COMPARATIVE EFFICIENCY OF MEDIA	TOTAL NUMBER OF MEDIA CONTAINING		
				S	$Na_2S_2O_3$	Neither S nor $Na_2S_2O_3$
A	9	11.2	Good	4	2	2
B	8	10.0	Fair	5	1	3
C	10	12.5	Poor	2	1	7
A + B + C	27	33.7	Fair	11	4	12
D	53	66.2	Negative	0	5	48

efficiency—3 per cent in group A, 5 per cent in B, 11 per cent in C, and 80 per cent in D. The addition of $Na_2S_2O_3$ was beneficial in about 44 per cent, since of the 9 media tested, 4 were positive and 5 negative. In toto, then 15, or 56 per cent, of the media showing growth contained S or $Na_2S_2O_3$. Of the 17 in the best groups, A and B, 12, or 71 per cent, contained S or $Na_2S_2O_3$.

Thus far, in our experience, no mention has been made in the literature of this seemingly beneficial effect of sulphur from the above sources on the metabolism of streptococcus. Of course, it is a well known fact that sulphur is an important and essential constituent of the organism. Nevertheless, the reason for the marked superiority of this substance over most others used is not clear. This finding may possibly be, though probably is not, merely accidental. It may be that streptococcus is able to utilize this element more readily than

some others; and also that when the nutrient supply is very low, as in synthetic media, this faculty becomes strikingly apparent. Or, it may be that sulphur reacts with some harmful products excreted by the organism, thereby saving it from more rapid decline. Further investigation is necessary.

2. *Effect of calcium compounds.* The media employed and the corresponding results are summarized below:

Set a

Medium 15b (0): 0.5% $(NH_4)_2CO_3 \cdot H_2O$; 1.5% NH_4NO_3; 0.0025% $CaCl_2$; 0.5% $MgSO_4 \cdot 7H_2O$; 0.5% K_2HPO_4; 0.05 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+ in the first subculture of the first transplant.

Medium 15b[3] (0): No $CaCl_2$; 0.06 cc. NaOH (2.123M) per 5 cc.; the remaining constituents of medium 15b. *No growth.*

Set b

Medium 27a (0): 0.2% aspartic acid; 0.0036% $MgSO_4 \cdot 7H_2O$; 0.1% K_2HPO_4; 0.04 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+, 2+, 2+, in the first three subcultures of the first transplant; +, 1c, in the first two subcultures of the second transplant.

Medium 27i (0): 0.5% $CaCl_2$; 0.046 cc. NaOH (2.123M) per 5 cc.; the remaining constituents of medium 27a. *Growth:* 2+, 2+, 2+, 2+, in the first four subcultures of the first transplant; +, +, +, +, in the first four subcultures of the second transplant.

Set c

Medium 27cVI (0): 0.2% aspartic acid; 0.025% $CaCl_2$; 0.0036% $MgSO_4 \cdot 7H_2O$; 1.0% KH_2PO_4; 0.1% K_2HPO_4; 0.083 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, 4+, 2+, 2+, +, in the first five subcultures of the first transplant; +, 4c, 2c, +, in the first four subcultures of the second transplant.

Medium 27cXI (0): 0.005% $CaCl_2$; 0.085 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 27cVI. *Growth:* 4+, 4+, 2+, in the first three subcultures of the first transplant; +, +, 1c, in the first three subcultures of the second transplant.

Set d

Medium 15 (21): 0.5% $(NH_4)_2CO_3 \cdot H_2O$; 0.05% $CaCl_2$; 0.5% $MgSO_4 \cdot 7H_2O$; 0.5% K_2HPO_4; 0.06 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+, 2+, 2+, in the first three subcultures of the first transplant; +, +, in the first two subcultures of the second transplant.

Medium 15a (0): 0.0025% $CaCl_2$; 0.075 cc. NaOH (2.123M) per 5 cc.; remaining constituents of Medium 15. *No growth.*

Medium 15a[2] (0): 0.0005% $CaCl_2$; 0.073 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 15. *No growth.*

Medium 15b (0): 1.5% NH_4NO_3; 0.05 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 15a. *Growth:* 2+ in the first subculture of the first transplant.

Medium 15b[2] (0): 1.5% NH_4NO_3; 0.065 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 15a[2]. *No growth.*

Set e

Medium 103 (6): 1.0% NH_4 lactate; 0.2% $Na_2HPO_4 \cdot 12H_2O$; 0.113 cc. NaOH (2.123M) per 5 cc. *No growth.*

Medium 104 (6): 1.0% CaCO; 0.003 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 103. *Growth:* 4+, 3+, in the first two subcultures of the first transplant.

The results show that Ca was always beneficial to some extent. Sets a and b indicate that the addition of $CaCl_2$ prolonged the life of the organisms. Sets c and d demonstrate that an increase in $CaCl_2$ increased the availability of the medium; Set e, that other Ca salts caused a similar rise in utilization.

3. *Effect of Na and K compounds.* Na in some forms seems to be injurious. Enough data have not yet been gathered to warrant a definite conclusion in this relation. The aim here is to give a preliminary report of the available data.

Medium 27k (0): 0.2% aspartic acid; 0.001% $FeSO_4 \cdot 7H_2O$; 0.0036% $MgSO_4 \cdot 7H_2O$; 0.1% K_2HPO_4; 0.038 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, 4+, 3+, 3+, 3+, 3+, in the six subcultures of the first transplant; 1c, 3+, 2+, 2c, in the first four subcultures of the second transplant.

Medium 27kI (0): 0.2% $NaNO_3$; 0.036 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 27k. *Growth:* 4+, 4+, 2+, in the first three subcultures of the first transplant; 1c in the first subculture of the second transplant.

Medium 229 (8): 1.0% aspartic acid; 0.4% $NaNO_3$; 0.158 cc. NaOH (2.123M) per 5 cc. *No growth.*

Medium 230 (8): 1.0% aspartic acid; 0.315 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.004 cc. NaOH (2.123M) per 1 cc. after sterilization. *Growth:* 3+, 2+, in the first two subcultures of the first transplant; 1c in the first subculture of the second transplant.

Medium 27a (0): 0.2% aspartic acid; 0.0036% $MgSO_4 \cdot 7H_2O$; 0.1% K_2HPO_4; 0.04 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+, 2+, 2+, in the first three subcultures of the first transplant; +, 1c, in the first two subcultures of the second transplant.

Medium 27h (0): 2.5% Na_2SO_4; 0.031 cc. NaOH (2.123M) per 5 cc.; remaining constituents of 27a. *Growth:* + in the first subculture of the first transplant.

Medium 196 (8): 1.0% glycocoll; 0.015 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, 2+, 3c, in the first three subcultures of the first transplant.

Medium 197 (8): 1.0% glycocoll; 0.2% NaCl; 0.015 cc. NaOH (2.123M) per 5 cc. *Growth:* 2+ in the first subculture of the first transplant.

Although an equivalent series for K salts cannot be reported at present, the following four sets of media (A–D) indicate practically no differences between Na and K. The results were obtained with organisms from stock cultures at different ages; one, I, was three months older than the other, II.

Set A

Medium 461 (13): 1.0% aspartic acid; 0.04% $MgSO_4 \cdot 7H_2O$; 0.7% malic acid; 0.25% Na_2CO_3; 0.2% $Na_2HPO_4 \cdot 12H_2O$; 0.001% $CaCl_2$; 0.32 cc. NaOH (2.26M) per 5 cc. *Growth:* For organism I—4+, 2+, +, 4c, in the first four subcultures of the first transplant; for organism II—3+, 3+, +, 2+, in these subcultures, and 4c in the first subculture of the second transplant.

Medium 518 (13): Na salts of medium 461 were substituted by K salts. 0.25% K_2CO_3; 0.2% K_2HPO_4; 0.43 cc. KOH (1.9139M) per 5 cc.; remaining constituents of medium 461. *Growth:* For organism I—4+, 3+, +, +, +, 2c, in the first six subcultures of the first transplant, and + in the first subculture of the second transplant; for organism II—4+, 2c, in the first two subcultures of the first transplant.

Set B

Medium 60 (8): 1.0% aspartic acid; 0.2% $Na_2HPO_4 \cdot 12H_2O$; 0.18 cc. NaOH (2.26M) per 5 cc. *Growth:* For organism I—4+, 3+, 3+, 2+, 3+, 2+, in the first six subcultures of the first transplant; for organism II—2+, +, in the first two subcultures of the first transplant.

Medium 212 (8): 1.0% aspartic acid; 0.2 cc. KOH (1.9139M) per 5 cc. *Growth:* For organism I—4+, +, in the first two subcultures of the first transplant; for organism II—3+, +, in these subcultures.

Set C

Medium 74 (1): 0.5% aspartic acid; 0.1% $MgSO_4 \cdot 7H_2O$; 0.1% K_2HPO_4; 0.122 cc. NaOH (2.26M) per 5 cc. *Growth:* For organism I—4+ in the first subculture of the first transplant; for organism II—3+, +, in the first two subcultures of the first transplant.

Medium 74a (1): 0.13 cc. KOH (1.9139M) per 5 cc.; remaining constituents of medium 74. *Growth:* For organism I—4+, 2+, +, in the first three subcultures of the first transplant; for organism II—3+, 2+, 4c, in these subcultures.

Set D

Medium 195 (21): 1.0% aspartic acid; 2.0% glycerol; 0.1% K_2HPO_4; 0.05% Na_2SO_4; 0.18 cc. NaOH (2.26M) per 5 cc. *Growth:* For organism I—4+, 2+, 2+, 2+, in the first four subcultures of the first transplant; for organism II—4+, 2+, 3+, 3+, in the first four subcultures of the first transplant, and 5c in the first subculture of the second transplant.

Medium 194 (21): 0.05% K_2SO_4; 0.22 cc. KOH (1.9139M) per 5cc. *Growth:* For organism I—4+, 3+, 2+, 2+, 2+, in the first five subcultures of the first transplant; 1c, 4c, in the first and third subcultures of the second transplant. For organism II—4+, 3+, 3+, 4+, in the first four subcultures of the first transplant; 4c, 3c, in the first and third subcultures of the second transplant.

4. *Effect of Fe compounds.* The results for Series a, b, and c, show a definite increase in vitality upon addition of $FeSO_4$ to the media, while Series d indicates the contrary. Although the ratio is 3:1 in favor of the former, we do not consider this sufficient evidence to support a definite opinion. The results suggest the probable tendency.

Series a

Medium 347 (7): 0.2% aspartic acid; 0.02% $MgSO_4 \cdot 7H_2O$; 0.02% KCl; 5.0% sucrose; 0.03 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.003 cc. NaOH per 1 cc. after sterilization. *Growth:* +, +, in the first and third subcultures of the first transplant.

Medium 346 (7): 0.001% $FeSO_4 \cdot 7H_2O$; 0.035 cc. NaOH (2.123M) per 5 cc. before sterilization; 0.002 cc. NaOH per 1 cc. after sterilization; remaining constituents of medium 347. *Growth:* 2+, 2+, +, 0, 2c, in the first five subcultures of the first transplant.

Series b

Medium 27i (0): 0.2% aspartic acid; 0.5% $CaCl_2$; 0.0036% $MgSO_4 \cdot 7H_2O$; 0.1% K_2HPO_4; 0.04 cc. NaOH (2.123M) per 5 cc. *Growth:* 4+, 3+, +, +, +, in the first five subcultures of the first transplant; +, +, in the first two subcultures of the second transplant.

Medium 27iII (0): 0.001% $FeSO_4 \cdot 7H_2O$; 0.04 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 27i. *Growth:* 4+, 4+, 4+, 4+, 4+, +, in the first six subcultures of the first transplant; +, 2+, +, +, +, in the first five subcultures of the second transplant.

Series c

Medium 27j (0): 0.2% aspartic acid; 0.0036% $MgSO_4 \cdot 7H_2O$; 0.05% KCl; 0.1% K_2HPO_4; 0.039 cc. NaOH (2.123M) per 5 cc. *Growth:* 3+, 3+, in the first two subcultures of the first transplant.

Medium 27jI (0): 0.001% $FeSO_4 \cdot 7H_2O$; 0.038 cc. NaOH (2.123M) per 5 cc.; the remaining constituents of medium 27j. *Growth:* 3+, 3+, 2+, 2+, in the first four subcultures of the first transplant; 0, +, 1c, in the first three subcultures of the second transplant.

Series d

Medium 27ii (0): 0.2% aspartic acid; 0.01% $CaCl_2$; 0.0036% $MgSO_4 \cdot 7H_2O$; 0.1% K_2HPO_4; 0.042 cc. NaOH (2.123M) per 5 cc. *Growth:* 3+, 4+, +, +, in the first four subcultures of the first transplant; + in the first subculture of the second transplant.

Medium 27iiI (0): 0.001% $FeSO_4 \cdot 7H_2O$; 0.038 cc. NaOH (2.123M) per 5 cc.; remaining constituents of medium 27ii. *Growth:* 2+, +, 2+, in the first three subcultures of the first transplant.

III. CONCLUSIONS

(a) Sulphur was exceptionally beneficial to streptococcus metabolism, in media containing only inorganic substances.

(b) Calcium salts were beneficial.

(c) Sodium salts were injurious.

(d) There were no discernible differences between sodium and potassium salts in similar media.

(e) Ferrous sulphate was generally beneficial, but also slightly harmful in some cases.

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A PHYSICO-CHEMICAL STUDY OF THE STRUCTURE OF DENTAL ENAMEL[1]

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I. MEMBRANE POTENTIALS

A. Introduction

A study of the potential differences that may arise across a membrane, when it separates two salt solutions of different concentrations, throws considerable light upon the structure and properties of the membrane under investigation. Many membranes, both living and non-living, appear to retard or prevent the movement through them of the ions of one electrical sign, while permitting the passage of ions of the opposite sign. As a result of this selective permeability, electrical potentials may arise whenever there exists an ionic-concentration gradient across a membrane. The magnitude of these potentials varies from membrane to membrane. In the more impermeable membranes the values may reach nearly the theoretical maximum, which, for a 10:1 concentration gradient at 20°C. is about 58 milli-

[1] A preliminary report of a portion of this study was presented at the Sixth General Meeting of the International Association for Dental Research: *Journal of Dental Research*, 1928, viii, p. 404.

volts.[2] More permeable membranes give lower values; very permeable membranes give very small potentials. The permeability of a membrane to salts may thus be at least roughly gauged by a determination of the magnitude of the potentials developed across it.

A number of investigators have carried out such measurements upon various animal, plant, and non-living membranes. Thus Loeb and Beutner (1, 2), working with the skin of the apple, have shown that membrane potentials approximating the theoretical maximum may be measured across this material. Michaelis and his associates (3) have studied collodion membranes of various grades of permeability, and correlated the potentials observed with the membrane structure. We have recently carried out a study of membrane potentials across the skin of the frog (4), in which we have found that the polarity of the potential depends upon the pH of the applied solutions; and have detected certain correlations between membrane charge and direction, and magnitude of membrane potential, which throw light upon the mechanism of the differential permeability to ions. These correlations will again be stressed in the present paper. A fuller bibliography of membrane potential studies is given in a former paper (4).

In the first section of the present communication are given some results of the study of membrane potentials across the enamel of canine teeth of dogs. In Sections II and III evidence of the permeability of tooth structures to water is presented. Preliminary reports of this work have been published (5, 6).

B. Experimental

Dog canine teeth, showing no imperfections or cavities when examined by a hand lens, were extracted within a few hours of the death of the animal. Care was taken not to injure the enamel in the process of extraction. The coronal portions of the teeth were sawed off at the cemento-enamel junction. Some of the teeth were then excavated with variously sized burs, to remove a maximum amount of dentine without fracturing the enamel. Others were studied without removal of the dentine. In a few teeth the enamel was removed

[2] When the "activity" of the solutions is allowed for, in the calculations, this figure is reduced to nearly 55 millivolts.

and the dentine studied alone. All of the preparations were placed in distilled water for a period ranging from a few days to two months, so that an opportunity might be afforded for washing out salts and any other soluble substances present in or on the structure.

At the conclusion of this period the enamel cup was placed into a salt solution of known concentration and composition, and allowed to remain there for from 24 to 48 hours, when it was arranged in the apparatus as shown in *fig. 1*. The enamel cup was sealed with dental

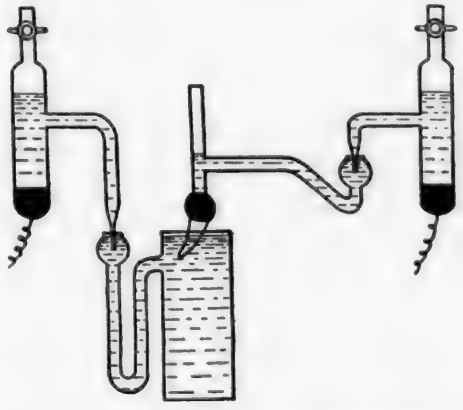

FIG. 1. DIAGRAM OF THE APPARATUS USED IN THE MEASUREMENT OF MEMBRANE POTENTIALS ACROSS ENAMEL

One solution is placed within the tooth and connected glass tube and side arm. The other, which may be changed at will, is placed in the lower vessel. Electrical connection to a potentiometer is completed through saturated KCl-calomel half cells. The tip of the tooth only is allowed to come into contact with the lower solution.

wax to the end of a glass tube. Any desired solution was then brought into contact with the inner surface of the enamel cup by filling this tube and the connected side-arm. The tip of the tooth was then partly immersed in an outside solution in the lower vessel. Electrical connection of the two solutions with a Leeds and Northrop portable potentiometer was completed through two calomel half-cells, as shown in the figure. Since the electrical resistance of such a system is high, it was necessary to use, as a null instrument, either a high

sensitivity moving-coil galvanometer, or a capillary electrometer. We have usually used the latter instrument.

The technique for the determination of membrane potential, when the salt involved is KCl, follows. After previous immersion in 0.1 *M* KCl, the enamel cup is mounted in the apparatus and brought into contact with portions of the same solution, which fill both inside and outside vessels. A reading of the potential difference in the system under these conditions is then taken. The concentration of the KCl of the outside solution is changed, in five-fold dilutions, ranging from 0.02 *M* to 0.0008 *M*, and differences of potentials noted for each dilution. At all times the concentration of KCl inside the enamel cup remains 0.1 *M*.

In the first instance, where the concentration of KCl is identical on both sides of the enamel, the P.D. observed is usually not more than a few millivolts, and often at or near zero. As the solution outside is progressively diluted, however, a P.D. appears, and rises to a higher value at each step in the dilution, the more dilute solution becoming more and more positive. Typical readings taken at 20°C. are these:

M concentration of KCl		P.D. (sign is that of outside solution)
Inside	*Outside*	*Millivolts*
0.1	0.1	+5
0.1	0.02	+25
0.1	0.004	+54
0.1	0.0008	+84
0.1	0.004	+48
0.1	0.02	+19
0.1	0.1	+5

Another similar series of values is shown graphically in *fig. 2*. In this figure there are also given readings taken upon the dentine of the same tooth after removal of the enamel, as well as readings obtained with an egg-shell. The more permeable structures give much lower P.D. values. The tooth without enamel is much more permeable than when enamel is present.

The values obtained on these various structures may now be compared with the theoretical maximum for an ideal membrane. In such

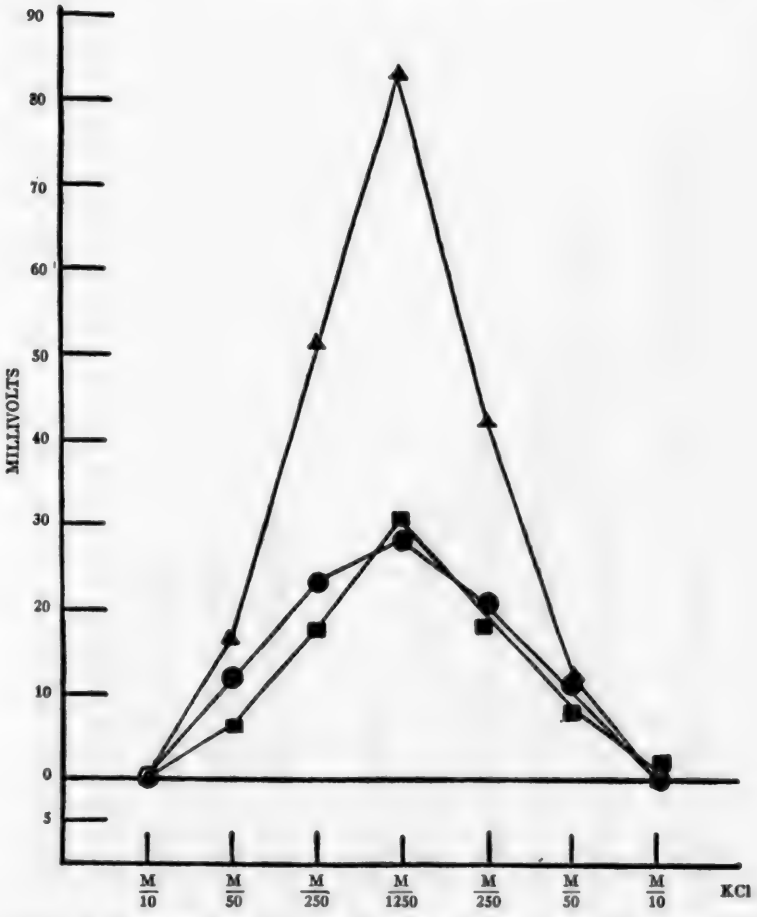

FIG. 2. GRAPH SHOWING DIFFERENCES IN THE MAGNITUDE OF MEMBRANE POTENTIALS OBSERVED ACROSS DIFFERENT STRUCTURES

The KCl concentrations shown are those of the outside solutions which, below 0.1 *M* KCl, become electropositive. In every case the inside solution is 1 *M* KCl.

▲—Concentration series for enamel

■—Similar series for dentine of the same tooth

●—Similar series measured across an egg shell

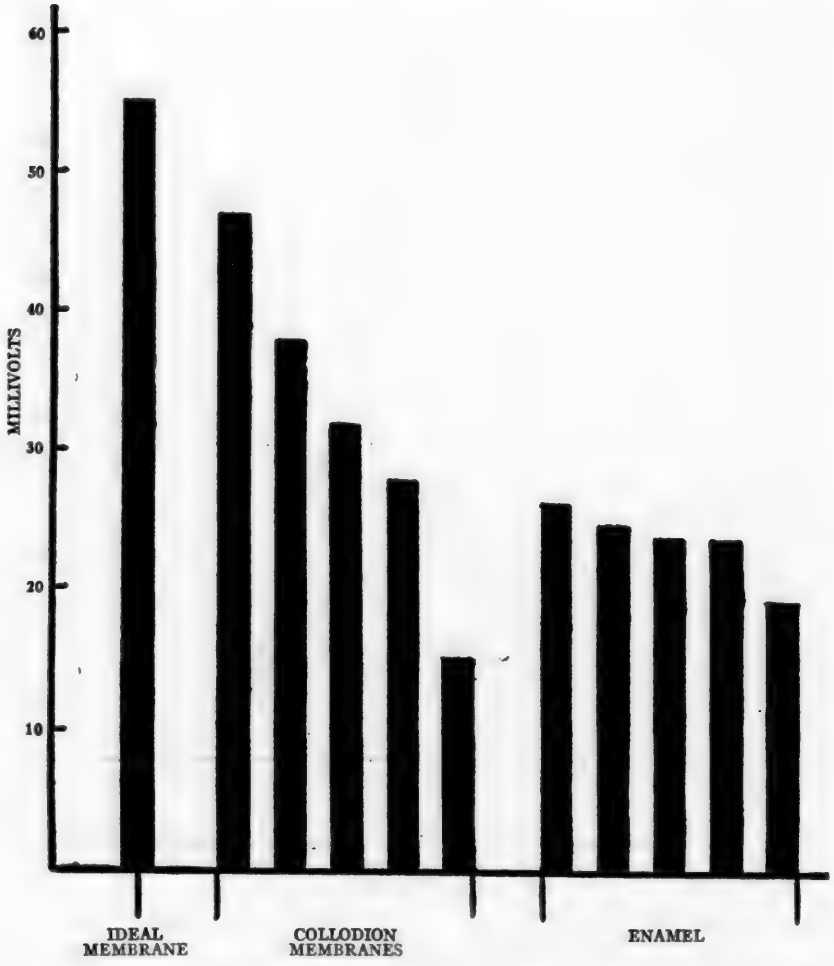

FIG. 3. VALUE OF CO. P. (SEE TEXT) FOR AN IDEAL MEMBRANE, CORRECTED FOR "ACTIVITY" OF THE SOLUTIONS, COMPARED WITH A SERIES OF VALUES MEASURED BY MICHAELIS ACROSS COLLODION MEMBRANES OF VARYING DEGREES OF PERMEABILITY, AND WITH A SERIES OBSERVED BY US ACROSS ENAMEL CUPS

an ideal membrane, which is permeable to one ion of the salt but impermeable to the ion of opposite electrical sign, the calculated P.D. at at 20°C. for a concentration gradient of 5:1 is 41 millivolts. The value of the P.D. across such an ideal membrane for any concentration gradient may be calculated from the formula developed by Nernst (7).[3] It was expected that the P.D. values across a structure so dense as that of enamel would closely approximate the theoretical maximum. They are, however, definitely less than that maximum. When, for a concentration gradient of 0.1 *M*—0.01 *M* KCl, the P.D. developed across enamel is compared with the calculated values derived for an ideal membrane, and with those found by Michaelis (3) across collodion membranes of varying degrees of permeability, the relationships graphically shown in *fig. 3* become evident. The enamel membranes resemble what Michaelis would call "poor" or "large-pored" collodion membranes. He has proposed to characterize membranes in terms of their membrane potentials for this particular salt and concentration gradient (0.1 *M*—0.01 *M* KCl), and speaks of the P.D. observed as the Co. P. of the membrane.[4] For enamel this value is of the order of 19 to 27 millivolts, or less than half the theoretical maximum. In a more dilute range of salt concentrations the P.D. values across enamel become larger, but have never, in our experience, risen above 45 millivolts for a 10:1 concentration ratio.

It may be inferred, therefore, that, under these experimental conditions, enamel membranes are not able *completely* to prevent the passage of ions of one electrical sign, but allow both positively and negatively charged ions to penetrate their structure. However, the movement of negatively charged anions is very much retarded in contrast to the comparatively free passage to positively charged cations. The latter tend to move through the membrane more rapidly, and give their positive sign to the more dilute solution.

Further observations on the membrane P.D. developed across enamel, in salt solutions other than KCl, substantiate these inferences

[3] For a more detailed mathematical treatment of this particular point, and for a more elaborate discussion of membrane potentials in general, the reader is referred to Freundlich (8) and Michaelis (9).

[4] "Co. P." is an abbreviation used by Michaelis to designate concentration potential between 0.1 *M* and 0.01 *M* KCl.

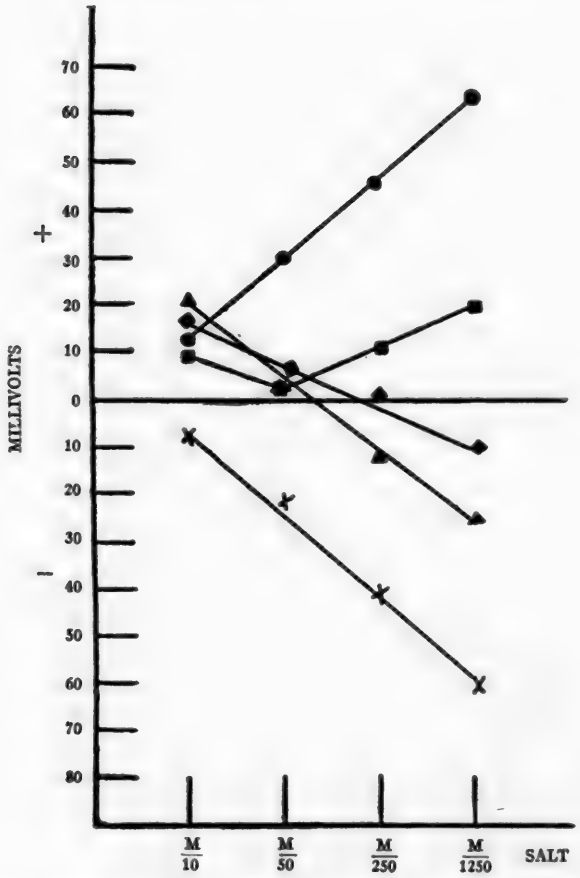

FIG. 4. GRAPH TO SHOW THE GRADUAL REVERSAL IN POLARITY OF THE MEMBRANE POTENTIALS WHEN KCl IS REPLACED BY $CaCl_2$

The sign is that of the dilute solution. In all cases the concentration within the tooth is 0.1 *M*.

●—Concentration series with KCl
■—Series with $CaCl_2$ one-half hour later
◆—Series with $CaCl_2$ 3 hours later
▲—Series with $CaCl_2$ 24 hours later
X—Series with $CaCl_2$ 48 hours later

as to the penetration of salt into the enamel. If the KCl bathing the enamel cup is replaced by $CaCl_2$, the concentration P.D. values obtained across the structure are gradually diminished; and, at the end of 24-hour immersion in $CaCl_2$, their polarity is reversed, the more dilute solution now being electro-negative.

In the following experiment an attempt has been made to follow the time relations of such reversals of membrane P.D. Typical readings were first secured from an enamel cup previously immersed in 0.1 *M* KCl and then brought into contact with a dilution series of KCl. The first series of readings are shown in *fig. 4* (●). In this particular case the initial reading was somewhat larger than was usually observed under these conditions, but the direction and magnitude of the changes in P.D. resulting from the application of the dilution series were fairly typical. Then, 0.1 *M* $CaCl_2$ was placed inside and outside the membrane, replacing the KCl. After an initial reading, a dilution series of $CaCl_2$ was applied as quickly as possible to the outside of the enamel, and the potential measured for each dilution, as shown in *fig. 4* (■). The enamel cup was then allowed to remain in contact with 0.1 *M* $CaCl_2$ on both sides for periods of 3, 24, and 48 hours. At the end of each period a determination of membrane P.D. was made, as shown in the figure. A definite reversal of polarity over the whole range is apparent in the series secured after 3 hours (♦), and becomes even more evident after 24 hours (▲), and after 48 hours (×).

It is of interest to note that the effect is reversible, i.e., when $CaCl_2$ is replaced by KCl, the polarity of the membrane P.D. gradually tends to reverse and at the end of 24 hours has definitely returned to its original direction. Solutions of the salts of other monovalent cations, such as NaCl, give results similar to those obtained with KCl.

The effect of the Ca^{++} in reversing the membrane P.D. is not specific, but is rather due to its valency, for the same effect was obtained with solutions of the chlorides of Mg^{++}, Ba^{++}, Sr^{++}, and even with Mn^{++} salts. Above a certain concentration, H^+ also produces a similar reversal, in a manner analogous to that observed previously on the skin of the frog (4). In acetate buffers this reversal point for enamel is at pH 4.3. The details of the experiments with other ions will be published elsewhere.

Such profound changes in the electromotive behavior of these enamel membranes suggest that the various ions are able to penetrate the structures slowly, and produce fundamental modifications in their physical characteristics. The nature of this modification will become much clearer after a consideration of the data in Section II.

II. ELECTROENDOSMOSIS

A. Introduction

An extensive array of experimental work shows that water in the pores of an organized membrane behaves as if it had assumed an electrical charge opposite in sign to that resident upon the pore walls themselves, and that, with an externally applied P.D., water may be made to pass through the structure of the membrane. The movement of water thus produced is spoken of as electroendosmosis. Among recent studies of this phenomenon may be mentioned those of Rein (11), who studied the effect in human skin, and of Mudd (10), who, working with pericardium and other similar membranes, observed a reversal of the direction of electroendosmotic transport of water when the solutions in contact with them were sufficiently acidified. Entin (12) has reported an electroendosmotic transport of water across enamel. We also carried out electroendosmotic measurements with skin of the frog (4).[5]

Several unsuccessful attempts were made to secure an electroendosmotic transport of water across enamel cups before we finally were able to secure the crude measurements here reported. The amount of water transported, even with high voltages, is, however, very small. We have been able to obtain only very rough qualitative indications of the direction of water movement under various experimental conditions, but these are sufficient to justify certain general conclusions.

B. Experimental

The electroendosmotic apparatus described by Mudd (10) was used. *Fig. 5* represents the arrangement of the apparatus. The current

[5] For a further discussion of the theory of this phenomenon the reader is referred to Freundlich (8).

was derived from a 110-volt line in the laboratory, to which were added B batteries up to 200 volts, whenever additional current was needed. The amount of water movement across the enamel was read upon a graduated capillary shown in the diagram. As in the membrane-potential studies, the seal between tooth and glass tube was placed well above the level of the outside salt solution, so that

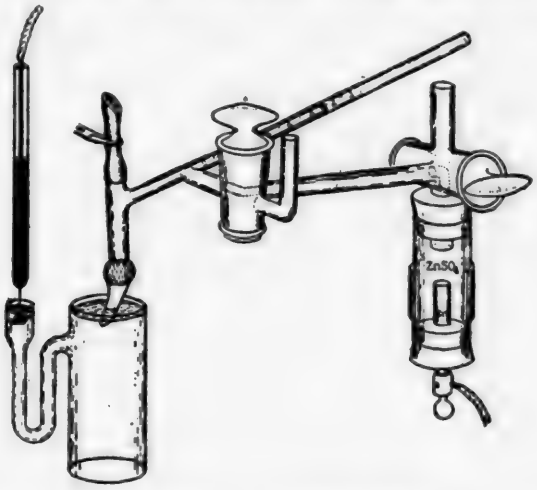

FIG. 5. APPARATUS FOR DETERMINING THE ELECTROENDOSMOTIC TRANSPORT OF WATER THROUGH THE ENAMEL CUP. (ADAPTED FROM MUDD)

The tooth is sealed to the end of a glass tube, which connects with a $Zn—ZnSO_4$ electrode. A solution fills the tooth and the connecting tubes, and comes up part way along the graduated capillary, upon which changes in volume may be noted. The tip of the tooth only is brought in contact with the outside solution. Electrical connection is completed through a platinum plate electrode dipping into the solution in the side arm of the lower vessel.

movement of water through the junction under the influence of the applied current was minimized. The surfaces of the seal, and of adjacent portions of enamel and glass, were touched from time to time with dry filter paper and the experiment discarded if any trace of moisture was detected. This test is, of course, unable to detect very small leaks, but if such were present, they would not have been large enough to affect the main movement of water through the enamel membrane.

In preparation for an electroendosmotic determination, the apparatus is filled with 0.2 *M* KCl so that the solution also fills the inside of the enamel cup. The stopcocks are then turned so that the inner surface of the enamel is in electrical connection with the $Zn-ZnSO_4$ electrode, through the KCl solution that fills the connecting tubes. The system is then entirely closed except for the capillary tube, which remains open to the air. Movements of water

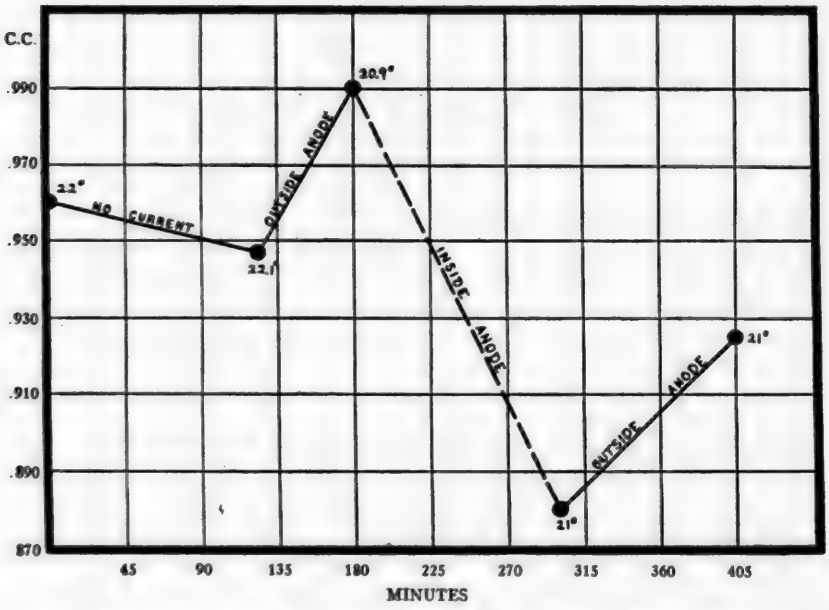

FIG. 6. ELECTROENDOSMOTIC TRANSPORT OF WATER ACROSS ENAMEL WHEN IMMERSED IN 0.2 *M* KCl

The water behaves as if it is positively charged. See *fig. 7.*

across the enamel under the influence of the applied P.D. are now investigated, the resulting change in volume of the inner solution being indicated by movements of the meniscus of water in the capillary tube in one direction or the other, and is read on the graduation on the capillary. The tip of the tooth is now brought into contact with another portion of the 0.2 *M* KCl solution, and electrical connections completed through a platinum electrode, as shown in *fig. 5*. Upon

the application of 200 volts to the two terminals, with the inner surface of the enamel cup connected to the cathode, the outer to the anode, a slow upward movement of the meniscus in the capillary tube is detected, indicating an increase in volume of the inner solution. This means that the water in the membrane behaves as if positively charged and moves through it toward the cathode. In *fig. 6*, where

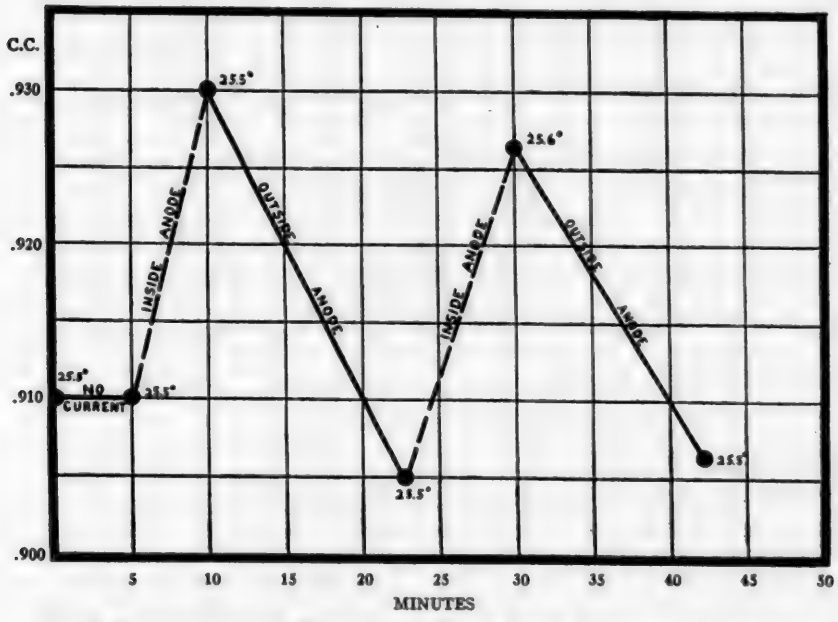

FIG. 7. ELECTROENDOSMOTIC TRANSPORT OF WATER ACROSS ENAMEL WHEN IMMERSED IN 0.2 *M* $CaCl_2$

The direction of movement is the reverse of that shown in *fig. 6*. The water now behaves as if it is negatively charged.

the position of the meniscus is plotted against time, this movement is represented by a sharp rise in the slope of the curve.

When the same P.D. was applied in the opposite direction the electroendosmotic transport of water was reversed, as shown in *fig. 6*. The water now flowed from inside to outside of the enamel cup, and the meniscus registered a decrease in volume within. Again the water behaved as if positively charged. It is therefore clear that

when the enamel is in contact with 0.2 *M* KCl it must bear a negative electrical charge upon its surfaces and pore walls, since the water in contact with it appears to assume the opposite electrical sign.

The KCl solution was now replaced with 0.2 *M* $CaCl_2$, and after several hours a second series of readings was taken. It at once

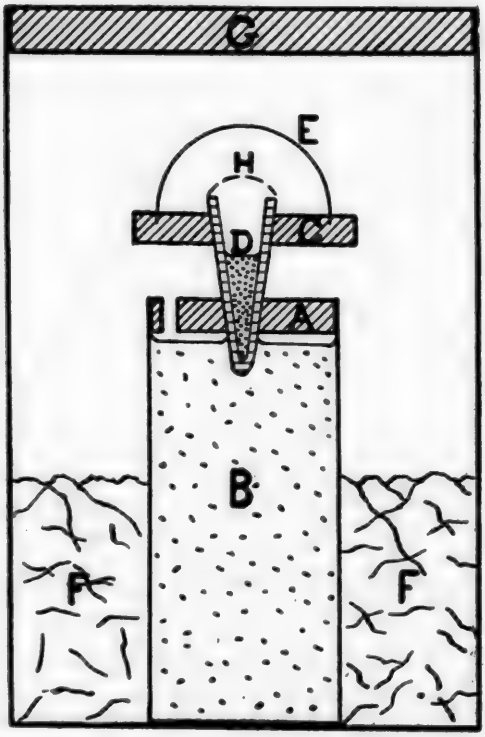

FIG. 8. DIAGRAM OF APPARATUS TO DEMONSTRATE MOVEMENT OF WATER ACROSS ENAMEL UNDER THE INFLUENCE OF AN OSMOTIC GRADIENT

See text for full description of the figure.

became evident that the direction of electroendosmotic transport of water had been reversed. Thus, to secure water movement from outside to inside, the inner surface of the enamel cup had now to be connected with the anode, the outer surface with the cathode. A reversal of this polarity reversed the direction of water movement.

Fig. 7 gives the readings obtained in this particular preparation. In both cases the water behaved as if negatively charged, and indicated that the charge on the membrane was positive.

In the presence of the monovalent K^+, therefore, the enamel bears a negative charge, but the divalent Ca^{++} is able to reverse this charge, presumably by adsorption of some of the Ca^{++} upon the surfaces of the structure. The correlation between these findings and the reversal of membrane potential by Ca^{++}, described in Section I, is evident. Its significance is discussed in Section IV.

These electroendosmotic determinations, while very rough, give a qualitatively correct picture. The movements of water observed, though small, were from 5 to 10 times the minimum quantity detectable by the method, which is about 0.005 cc. Temperatures were read from time to time, and are noted in the figures. It will be seen that, even where small fluctuations were present, the movement of water is not correlated with the temperature change. For the data in *fig. 7* the temperature was constant to 0.1°C., yet water movements appeared, definitely correlated with the polarity of the applied P.D.

III. OSMOSIS

Several investigators have reported that they could demonstrate the movement of water across the enamel in response to an osmotic gradient. Bunting (13) and, more recently, Livingston (14) and Entin (12) have described experiments in which they show that water may thus pass. We have repeated some of this work and find that experiments of these workers are open to criticism on a technical basis. Thus, Bunting sealed teeth to a glass capillary tube with sealing wax or paraffine, filled the tube and pulp chamber with NaCl solution, and then immersed the preparation in distilled water, which was allowed to rise above the seal itself. At the end of several hours a rise of fluid within the capillary was observed, and the application of the silver nitrate test to the water outside showed there the presence of chloride. We have no doubt that some of the movements of water and salt thus observed did indeed occur across the enamel, but we have always found, in similar experiments of our own, that the major part of the transfers occur through narrow spaces between seal and tooth, or seal and glass. In our experience it is exceedingly difficult, if not

impossible, to prevent a slow leak of water and salt through such crevices, and we have not been successful in devising a seal that is entirely impermeable under water. The presence of minute leaks, under the most carefully applied seals, can easily be shown by adding a small amount of $AgNO_3$ to the distilled water, not at the conclusion of the experiment, but at its beginning. If an NaCl solution is placed within the tooth and capillary, and the lower portion immersed so that the outside solution rises above the seal, fine streamers of AgCl are soon observed in the outside solution, particularly from the junctional line between seal and enamel surface. So much salt comes through and, presumably, so much water is able to pass in the opposite direction, that, in our opinion, the experiment is entirely unsatisfactory and inconclusive. The experimental arrangements of both Livingston and Entin appear to be open to similar objections.

After many unsuccessful attempts to secure an adequate seal, a different type of experiment was devised. We have found that osmotic movements across enamel can be demonstrated quite conclusively without sealing the tooth to any other tube. *Fig. 8* represents schematically the apparatus we have used for this purpose. With the tooth arranged as shown in the figure the only possible way in which water can pass to the inner side ot the enamel cup is through the enamel structure itself. The tip of the tooth is set through a paraffin block *A*, so that it comes into contact with distilled water in vessel *B*. A second paraffin block *C*, is set around the tooth at a higher level, just below the dentino-enamel line, so that the edges of the enamel cup project slightly above it. The tooth is now partly filled with a salt solution, usually *M* or saturated KCl, at level *D*. A hemispherical glass cover, *E*, is carefully sealed into the paraffin so as to cover the open end of the enamel cup. The whole preparation is placed within a larger vessel, and anhydrous $CaCl_2$ (*F*) is packed in around vessel *B*. Finally, the top of the larger vessel is closed with the paraffin seal, *G*. In such an arrangement, water movement along the outside surfaces of the enamel is not likely to occur, since all water vapor in the outer vessel is being continually removed by the $CaCl_2$, and a ring of enamel, between *A* and *C*, is exposed to this dry atmosphere. If water passes at all, it must pass through the enamel.

For several days after the completion of such a preparation, no

change in the level of water within the tooth was detected. As time elapsed, however, the level was seen to rise, and water movement became evident. After 3 to 5 weeks, the solution within actually filled the whole of the enamel cup and the meniscus was seen above its edges. Finally, so much liquid passed that the meniscus assumed the convex form indicated by the dotted line, *H*, of the figure. The passage of salt in the other direction was demonstrated by the addition of $AgNO_3$ to the solution outside the tooth.

The experiments of Bunting appeared to indicate that the enamel is a very permeable structure, since the transport of both water and salt was comparatively rapid. When the technical errors which we have discussed are eliminated, these movements become exceedingly slow, indicating a very much less permeable structure than his experiments seem to show.

IV. DISCUSSION

In the preceding sections we have presented experimental evidence bearing upon the structure of the enamel of a dog's canine tooth, and its permeability to salts and water. It was shown that a very definite correlation exists between the membrane potential measurements and the electroendosmotic determinations. In the presence of KCl, the enamel structure is found to bear a negative electrical charge. When in contact with solutions of this salt the observed membrane potentials indicate that the positively charged cations are able to penetrate the structure much more readily than do negatively charged anions. The structure therefore appears to retard the movement through it of ions bearing the same electrical sign as that resident upon the surfaces and pore walls of the enamel itself. In $CaCl_2$, the electroendosmotic readings indicate a positively charged structure; and, in correlation with this reversal in membrane charge, the membrane potentials observed with a concentration series of $CaCl_2$ solutions, are also reversed in polarity. This reversal of polarity indicates that the negatively charged anions are now able to penetrate more readily than are the positively charged cations. Once again the enamel retards the movement through it of ions bearing the same electrical sign as itself. We have made similar observations on the membrane potentials and membrane charge of skin of the frog, re-

versing an originally negative charge, not by the use of a divalent cation, but by increasing the concentration of the H^+ (4).

This correlation between membrane charge and membrane potential probably arises as a result of electrostatic effects between charged surfaces and ions of the same electrical sign nearby in the solution. As a result of an electrostatic repulsion, such ions are hindered from entering the narrow channels in enamel through which ionic movements are possible, whereas the ions of opposite sign are able to penetrate more freely. When a salt-concentration gradient exists across the enamel, these more freely moving ions, tending to diffuse from the more concentrated solution, give their electrical sign to the dilute solution, and a membrane potential appears. We thus find a simple physical explanation for the selective permeability of enamel and of other membranes for ions of opposite sign.

We are not at present able to state whether this physical characteristic of enamel has practical importance. There might appear to be the possibility that the penetration of the positively charged H^+ and the resulting decalcification of the enamel, could be hindered or prevented by a device that would render the membrane charge electropositive. In life it appears to be invariably electronegative, since concentration-potential studies carried out on newly extracted teeth of the dog, without any preliminary washing in distilled water, give P.D. values in which the dilute solution is electropositive. We may therefore infer that the membrane is itself negatively charged. The recent studies of Thurlow and Bunzell (15), and of Reeves (16), appear to show that the enamel is not attacked by the H^+ until the pH falls considerably below 3. At this pH the enamel is almost certainly positively charged, since we have found a reversal of membrane potentials across enamel cups at about pH 4.3. If our previous argument is correct, the movement of the positively charged H^+ into the enamel structure, on the acid side of this isoelectric point, is hindered by the membrane charge itself, at least over a considerable range. At or near molecular ionic concentrations, whether of acid or of salt, the whole group of electrokinetic phenomena, including cataphoresis, electroendosmosis, and concentration potentials across membranes, is suppressed. In molar solutions of acid, therefore, the "electrostatic screening" effect which we have here discussed must

vanish. In a higher pH range, from about pH 1.0 up to the isoelectric point, the effect should be present. There seems to be the bare possibility that such an effect might be responsible in part for the relatively slow decalcification of the structure in this pH range under conditions such as Reeves has described, but no definite statement can be made until actual experimental evidence on the point is at hand.

We must now consider the problem concerning the nature of the channels through the enamel which may permit the passage of water and salts. It will probably have been recognized that our data give information concerning the physical characteristics of the enamel structure as a whole, and do not indicate any conclusions as to the actual pathways along which substances may move. There is the possibility that all of the effects here described are crack and fissure phenomena, although we have carefully selected our material, and are reasonably sure that no gross defects of this sort were present. Fish (17) has described the presence of lamellae of organic material in dog teeth extending from the dentin to the surface of the enamel. Bödecker (18) has observed similar lamellae in human enamel. Such organic inclusions, however, take up a very small percentage of the total surface, and are, we believe, hardly sufficient to account for such striking effects as the complete reversal of a membrane P.D. with divalent cations. It is more probable that the movements of salt and water take place in the main along the organic matrix between the enamel rods, which Malleson (19) and Bödecker (20) have described, whose permeability to stains Fish (17) has studied, and whose organic nature has been conclusively established by the histo-chemical studies of Bödecker and Gies (21). The latter workers detected the presence in it of protein, which Rosebury and Gies (22) have shown is keratin. We have argued (4) that the reversal of membrane potentials across frog skin, as the pH is lowered, indicates that an amphoteric substance, probably protein in nature, is responsible for the electromotive behavior of the skin. Our observation of a similar reversal of potentials across the enamel, at an isoelectric point within the protein range (pH 4.3 in acetate buffers), is consistent with the view that here also a protein constituent of the structure is at least mainly responsible for the membrane charge, and through it, for the development and

polarity of the concentration P.D. and for the regulation of electro-endosmotic transport of water across the enamel. We cannot, however, assert that this view is certainly correct, since we have some evidence that pure tricalcium phosphate may show a similar behavior when the pH is varied (cataphoresis readings). This matter must therefore remain in doubt for the present. In any case the correlation between membrane potential and membrane charge is definite, even though the chemical substance responsible cannot yet be identified with certainty.

The observations here reported brings further support to the view that enamel is permeable for both salts and water, supplementary to that already obtained by histological studies. We are collecting further evidence on the matter, through studies of the changes in electrical conductivity of the enamel that can be demonstrated when a salt solution is applied to enamel previously washed through many changes of distilled water. The rate at which salt enters the structure can be easily studied by this method. The changes observed are so large that a penetration of salt involving the whole surface of the enamel is indicated. These studies, when completed, will be reported in a later communication.

V. SUMMARY

1. Membrane potentials may be demonstrated across the enamel of the canine teeth of dogs when the structure is made to separate two salt solutions. When the salt-concentration gradient is 0.1 *M* −0.01 *M* KCl, the P.D. values observed range between 19 and 27 millivolts, the dilute solution being electropositive. This is less than half the theoretical maximum calculated for an ideal membrane completely impermeable to the ion of one electrical sign. Even in more dilute solutions the P.D. values observed for a 10:1 concentration gradient do not rise above 45 millivolts, whereas the theoretical maximum at 20°C. is 58 millivolts. Such values for enamel indicate that it permits both ions of the salt to penetrate its structure, but very much retards the movement of negatively charged anions.

2. When solutions of $CaCl_2$ or of the chlorides of other divalent cations are used, instead of solutions of monovalent salts, the polarity of the dilute solution is electronegative. This reversal is fully accom-

plished only after some hours in these solutions, indicating a slow penetration of the salt into the enamel, the physical condition of which is profoundly modified, so that it now retards the movement of positively charged cations.

3. The nature of this modification is made clear by electroendosmotic determinations. It is found that water can be made to pass through enamel slowly under the influence of an applied P.D. In a 0.2 *M* solution of KCl the direction of flow indicates that the water behaves as if positively charged; the membrane must therefore bear a negative charge. In a 0.2 *M* solution of $CaCl_2$ the water behaves as if negatively charged; the membrane must therefore bear a positive charge, presumably as a result of the adsorption of some of the Ca.

4. When the membrane potential and electroendosmotic readings are considered together, it is evident that the enamel membranes, whether positively or negatively charged, tend to retard the movement into them of ions of the same electrical sign as they themselves bear. It is suggested that this is a simple electrostatic effect, whereby a charge on the surfaces and pore walls of the enamel tends to repel ions of like sign, and hinder their entrance into the membrane, allowing oppositely charged ions to enter more freely.

5. The slow movement of water across enamel as the result of an osmotic gradient is also demonstrated, under conditions free from technical defects in previous work.

6. These observations do not permit the identification of the pathways through the enamel structure by which water and salts penetrate. It is suggested that the movements probably occur along the organic matrix between the enamel rods described by Malleson and by Bödecker.

We wish to express our appreciation to Mr. Samuel Fels of Philadelphia, and to the Philadelphia Academy of Stomatology, for their generous financial support.

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THE FATE OF THE AMELOBLASTIC CELLS OF THE ENAMEL ORGAN

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I. INTRODUCTION

Since Nasmyth (1839) first described the enamel cuticle it has received a great deal of attention from those interested in the investigation of dental structures. This interest, which has resulted in a prolonged discussion of its origin, structure, and function, has arrived at remarkably little agreement regarding the nature of the organ. Practically all the tissues in the region of the forming enamel, including the enamel itself, have been assigned parts in the structure of the enamel cuticle by the various investigators.

The main points at issue were reviewed by Mummery (1924). They were again stated by Neuwirth (1925), by Apituley (1925) in his monograph on Nasmyth's membrane, and by Chase (1926). Neuwirth believes that the enamel cuticle consists of a single membrane, and that it has its origin in the transformation of the ameloblastic layer of the enamel organ. Apituley states that Nasmyth's membrane is formed by the hornifying of the enamel pulp cells. (The present writer is uncertain as to just what cells are meant here.) He also states that the membranes may either have a structure or be structureless, depending upon the extent and degree of hornification of the cells involved. Chase has tabulated the principal conceptions regarding the origin and structure of Nasmyth's membrane, together with the names of the chief exponents of these various theories. He concluded

that "Nasmyth's membrane, in its most complete form, is derived from the remains of the entire enamel organ, excepting the enamel pulp, together with overlying layers of cells from the gingival epithelium."

The present paper deals only with the rôle that the ameloblastic layer of the enamel organ plays in the formation of the enamel cuticle as observed in erupting teeth in dogs. It will not concern itself with other structures that may or may not take part in the formation of the enamel cuticle. Neither will the matter of the function of the enamel cuticle nor the question of its persistence during the life of a normal tooth be considered here.

II. TECHNIC

Although other methods were employed, the following one yielded the most convincing results in the study of this structure. The formalin-fixed specimens of both hard and soft tissues were ground on a lathe with a rapidly cutting carborundum stone. No attempt was made to decalcify the hard substances nor to embed the soft parts. The specimen was held against the stone with a finger and a small stream of water played on it in order to prevent heating. The grinding was continued in one direction away from the enamel surface, until a desired thinness was obtained. The sections were then washed, stained with hematoxylin-eosin, and mounted. Extreme thinness could be sacrificed, since it was only necessary to study cell layers and groups and not individual cells. Difficulty arises in controlling the grinding as the sections approach the desired thinness. One can, of course, sacrifice all parts of the section not wanted for a particular study. Obviously many sections are spoiled in the attempt, but very good results may be obtained by persistence.

Better results are to be had after the coarse grain of the carborundum stone is somewhat worn and filled in with the grindings. Too much tearing is encountered in the use of new coarse stones.

This method has the advantage of being extremely simple. Very little preparation of the structures to be studied is necessary, and consequently there is less disturbance of the important relationships of hard and soft tissues. The use of the method is, of course, limited to certain studies.

III. OBSERVATIONS AND DISCUSSION

Fig. 1 shows a section of a permanent lower central incisor of a dog (*Canis familiaris*), prepared as described above. This tooth was still in the process of eruption in the living animal, approximately one third of the crown having emerged through the oral mucous membrane. The illustration shows the entire lingual surface covered with epithelial cells, the break in the incisal third representing a mechanical artefact.

The enlargements of the regions indicated on this surface show (*a*) normal-looking ameloblasts in the cervical third, (*b*) flattened epithelial cells in the incisal third, and (*c*) transitional forms in the intervening region of the enamel surface.

The individual cells in *fig. 2* (*1-A*), formerly of the ameloblastic layer of the enamel organ, stand out quite clearly when one takes into consideration the fact that this is a ground section. The typical columnar form and arrangement of these ameloblastic cells is clearly shown. It is to be observed that this layer is distinct from the overlying cells, which apparently are derived from other parts of the enamel organ. If the ameloblasts have not already ceased to deposit enamel they must at least be rapidly approaching the end of the period during which they function as ameloblasts. Note, however, that this preparation suggests that enamel may still be laid down in the cervical portion of the tooth after its incisal portion has appeared in the oral cavity. *Fig. 3* (*1-B*) shows ameloblasts that have lost to some extent their regular form and arrangement in the middle third of the lingual surface of the same tooth. One is inclined to conclude that these cells are now through with their function of enamel deposition. In *fig. 4* (*1-C*) the columnar character of the ameloblasts is fast disappearing, and in *fig. 5* (*1-D*) these cells have taken on a decidedly irregular polygonal form. In the final fig. (*6: 1-E*), which represents a region near the incisal end of the crown, the cells of the ameloblastic layer have become still further modified, adapting a definite squamous form and merging with the overlying cells, thus contributing to the enamel cuticle of the erupting tooth.

In this series of photomicrographs we are able to observe clearly the fate of the ameloblastic cells in their continuous transition from that of normal appearing ameloblasts in the cervical portion of

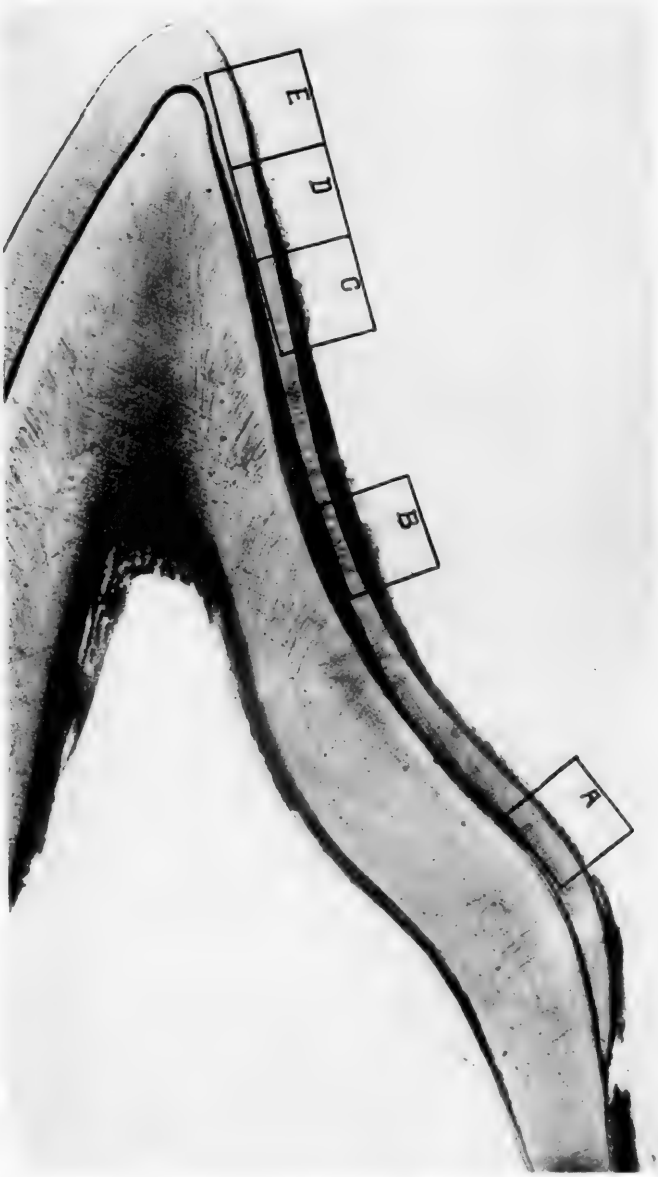

Fig. 1. Erupting lower incisor, *Canis familiaris*. Indicated regions *A*, *B*, *C*, *D*, and *E* are shown in higher magnification in *figs. 2–6*, successively. (× 15)

Fig. 2 (1-A). Ameloblastic layer on the enamel surface in the unerupted cervical third. (× 175)

Fig. 3 (1-B). Modified ameloblastic layer. (× 175)

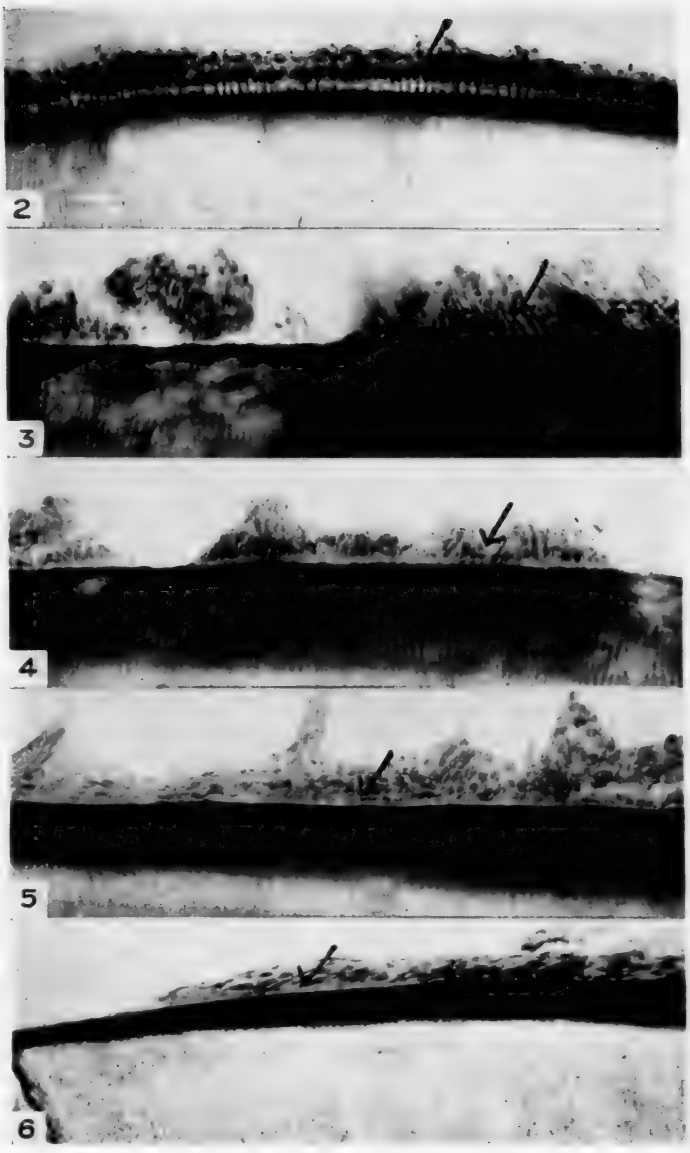

Fig. 4 (1-C). Progressive modification of ameloblasts towards squamous cell type. (× 175)

Fig. 5 (1-D). Ameloblasts further modified to polygonal forms. (× 175)

Fig. 6 (1-E). Finally modified ameloblast merging with the superimposed cells to form enamel cuticle. (× 175)

the erupting tooth of a dog, to flattened squamous cells in the recently erupted incisal portion of the same tooth. This observation having been made repeatedly, one may conclude that the modified ameloblastic layer contributes to the enamel cuticle of the newly erupted tooth.

IV. SUMMARY

(1) The ameloblastic cells of the enamel organ can still be found, apparently in a normal condition, in the teeth of dogs which have erupted through the oral mucous membrane.

(2) These cells can be found in all transitional forms, from the normal ameloblasts to flattened squamous epithelial cells, in passing from the cervical to the incisal portion of the crown.

(3) Definite evidence of morphogenic continuity of the ameloblastic cells of the enamel organ with a portion of the enamel cuticle of newly erupted teeth is presented.

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THE PENETRATION OF THE TISSUE BY VINCENT'S ORGANISMS

A Report of a Case[1]

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The history of the following case shows that the tissues may harbor the causal agents of Vincent's disease long after acute symptoms have subsided:

Mr. H.; age 35. The patient complained of severe pain in the gums and of all other symptoms indicative of Vincent's infection. On examination the mouth revealed a classic picture of the disease. The gums were markedly reddened and inflamed; and between the lower central incisors, and the lower right cuspid and first bicuspid, there were characteristic membranous lesions. Smears made from these areas showed Vincent's spirochete and Bacillus fusiformis in profusion, almost to the exclusion of all other bacterial forms (*fig. 1*). Under routine treatment, which in this case was the application of sodium perborate and the home use of this drug as a mouth wash, and the local application of methylene blue, the disease was checked in forty-eight hours. Within this time almost all pain had disappeared and the patient was entirely comfortable. Four days later all soreness was gone from the gums and there were no membranous areas, although the gums in the region where the membranes had been were still injected (*fig. 2*). There was considerable destruction of the tissue, especially on the lingual surface between the lower incisor teeth (*fig. 3*). Within ten days there was no evidence that the patient had ever had the disease.

So much for the case itself. I had often wondered why cases of Vincent's gingivitis had acute lapses long after they had been pro-

[1] Received for publication on June 13, 1929, which is noted here at the author's request. —[*Ed.*]

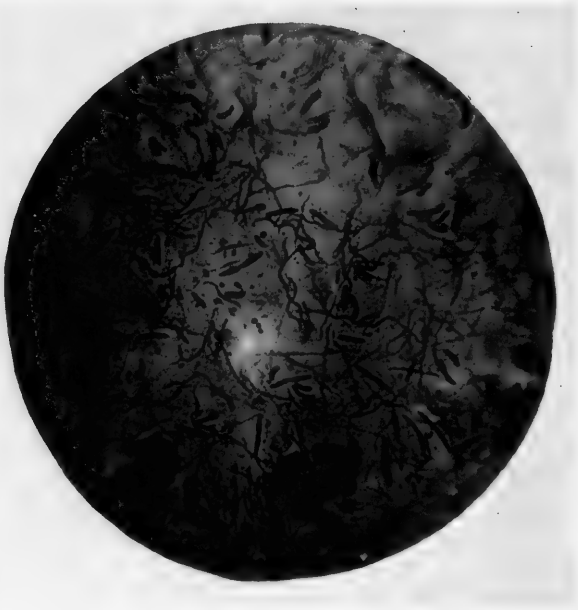

FIG. 1. A PHOTOMICROGRAPH OF A SMEAR MADE FROM A MEMBRANOUS AREA

Note the presence of characteristic spirochetes and Bacilli fusiformis, and the absence of all other bacterial forms.

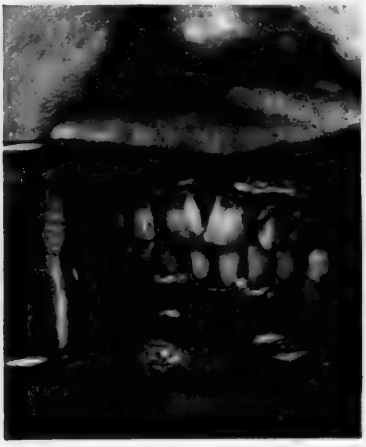

FIG. 2

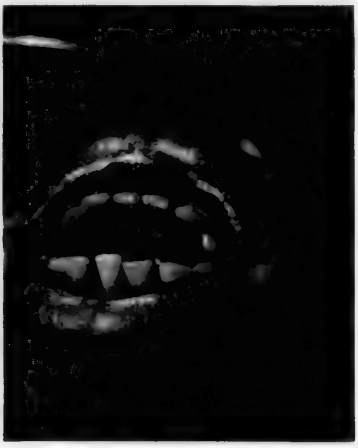

FIG 3

FIG. 2. FOUR DAYS AFTER THE BEGINNING OF ROUTINE TREATMENT

All acute symptoms have disappeared. One may note the gingivitis still present and the marked destruction of tissue between the lower central incisors, and between the lower right (patient's right) cuspid and bicuspid. See *fig. 3.*

FIG. 3. ANOTHER VIEW FOUR DAYS AFTER THE BEGINNING OF ROUTINE TREATMENT (*fig. 2*)

Shows the destruction lingually in the lower incisal region.

nounced well; or why, although no acute symptoms developed, there would remain inflamed and hemorrhagic areas. It occurred to me that perhaps the causal organisms had penetrated the tissue, a condition not previously mentioned in connection with Vincent's disease

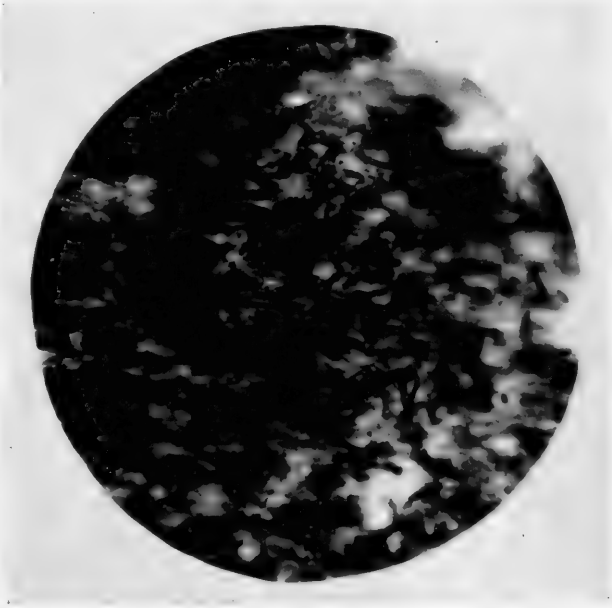

FIG. 4. A PHOTOMICROGRAPH OF THE SECTION OF GUM REMOVED

One notes the abundance of fusiform bacilli. This tissue was removed about ten days after all symptoms had disappeared. The gum looked normal, there was no pain, and to all appearances the patient was cured. There are few spirochetes present, but very many fusiform bacilli. These organisms have penetrated the section of the tissue nearest the root or that portion of the tissue lining the pocket. The sections were stained by the Levaditi method, which stains the organisms black against a brown background. These colors do not lend themselves readily to differential photography. Consequently, the contrast of the organisms against the background is difficult to bring out in a photomicrograph.

of the gums. Siegmund and Weber (3) show a drawing of a section of cheek mucous membrane disclosing mouth spirochetes and fusiform bacilli; it is interesting to note, but this section was made from a case of noma. Because of possible penetration, all the organisms might

not have been entirely eradicated in such instances as those I have mentioned. Accordingly, in the patient whose case is described above, I removed a piece of gum from between the lower right cuspid and first bicuspid for examination. This operation was done ten days after all symptoms had disappeared and the gum had clinically returned to normal. The excised tissue was prepared by the Levaditi method and examined under the oil-immersion lens. The tissue was so cut that it could be examined from without inwards, or from the epithelium toward the root of the tooth.

The examination brought out an interesting condition. There were numerous fusiform bacilli and some spirochetes within the tissue; and in that part nearest the tooth, or lining the pocket, the organisms had penetrated quite a distance. It was interesting to note that there were scarcely any spirochetes but a great many fusiform bacilli (*fig. 4*).

In 1920 I said that, "if a smear is made from a true case of Vincent's gingivitis, the spirillum of Vincent and the Bacillus fusiformis will be found to the almost total exclusion of all other forms of organisms" (2). This opinion I believe still holds true. Appleton recently made a similar observation (1). We may note from *fig. 1* how characteristic is the smear.

The finding of the Vincent organisms in the tissue, after all clinical conditions had disappeared, leads me to change an opinion I stated in the paper from which I just quoted. I then said that "these organisms have very little power to invade the tissues." In the light of my present findings this remark is untrue. More research is necessary in this relation. I offer this as a preliminary report to show the possibility of penetration. If future research proves that penetration occurs, a possible cause for acute remissions will be had, as well as one for continued chronicity. This would of course lead to a change in therapy.

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EARLY HUMAN TOOTH DEVELOPMENT, WITH SPECIAL REFERENCE TO THE RELATIONSHIP BETWEEN THE DENTAL LAMINA AND THE LIP-FURROW BAND[1]

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I. INTRODUCTION

History. The first records of the study of teeth go back to those of Aristotle, who observed variations in dentition among different animals. There is a long gap of no progress during the middle ages until 1562, when Eustachio found that the teeth came from the oral mucous membrane just as nails and hair came from the skin. In 1678 Leeuwenhoek studied the structure of enamel and dentine under the microscope.

The important studies of tooth development have been made since the last half of the nineteenth century. In Germany, such embryologists as v. Kölliker (1861–1865), Kollman (1869), Waldeyer (1871), and Hertwig (1874), were interested in tooth development In France, Lagros and Magitot (1865) investigated the development of teeth, and G. V. Black repeated their work in 1880. Röse (1891) made a careful study of dental embryology, and was the first to

[1] With the exception of two photographs taken from human material prepared at the Histology Laboratory of the College of Dentistry of the University of Illinois, this study is based on uninterrupted serial sections in the human embryological collection of the Department of Anatomy of the University of Chicago, and was made possible by the kind coöperation of Dr. G. W. Bartelmez, who made this material available and encouraged this investigation.

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reconstruct various stages of human tooth development in wax models. Among the more recent investigators are Leche, Adloff, Bolk, Dependorff, Ahrens, and Hopewell Smith. Very recently Orban (1928) made a thorough study of human tooth development, and reconstructed a number of representative stages in wax models.

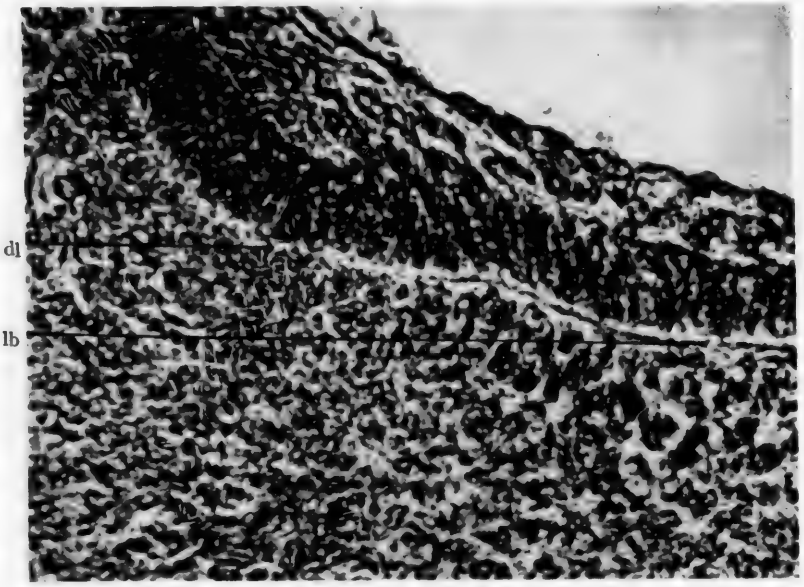

FIG. 1. TRANSVERSE SECTION OF A 21 MM. HUMAN EMBRYO (H 405, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 580

Early differentiation of dental lamina and lip-furrow band in the region of the future lower first premolar. *dl*—dental lamina. *lb*—lip-furrow band.

Dental lamina. During the sixth week of embryonic life there arises an epithelial thickening near the free margin of the jaws, which is continuous and runs arch-like along the entire extent of the jaws. It represents the Anlage of the ectodermal portion of the tooth and the epithelium of the gingivae, and is called the dental lamina. Histologically it consists of superficial squamous cells and a basal layer of columnar cells that belong to the stratum germinativum and are separated from the mesoderm by a distinct basement membrane.

Lip-furrow band. Shortly after the appearance of the dental lamina there arises between it and the future region of the lips and cheeks another but less prominent epithelial thickening. This thickening is, therefore, parallel and labial or buccal to the dental lamina, and represents what the Germans named the "Lippenfurchenleiste," or the lip-furrow band (*fig. 1*). It will later form the vestibule of the mouth (*fig. 6*). The tissue labial and buccal to the lip-furrow band

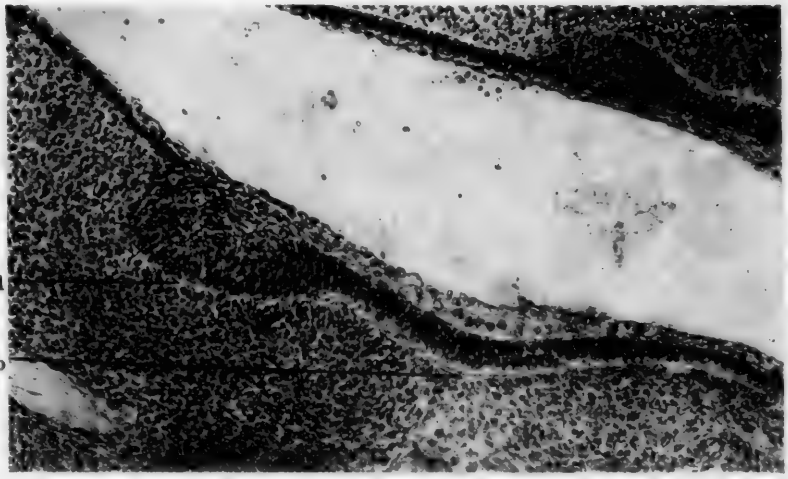

FIG. 2. TRANSVERSE SECTION OF A 21 MM. HUMAN EMBRYO (H 405, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 200

Bud-like proliferation of dental lamina to form tooth germ of lower labial incisor. *dl*—dental lamina. *lb*—lip-furrow band.

will develop into the lips and cheeks. The tissue immediately lingual to it will differentiate into the alveolar ridge (*fig. 6*).

Early stage of tooth germ. At ten points, corresponding to the future position of the deciduous teeth, the dental lamina extends more rapidly and reaches deeper into the mesoderm than elsewhere, with the result that these areas of more rapid growth increase in size, become bulbous at their deep end, and incline somewhat lingually (*fig. 2*). The dental lamina thus becomes scalloped at its deep surface. According to Orban the dental lamina does not grow down

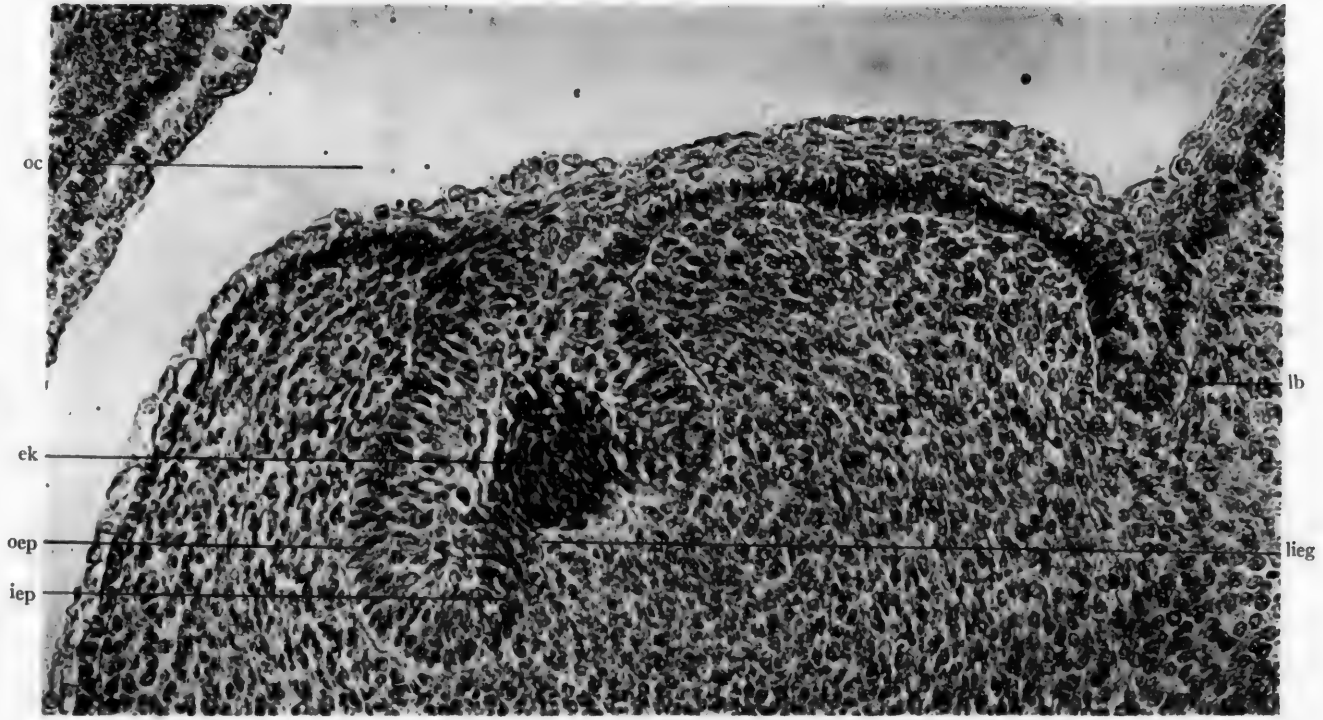

FIG. 3. TRANSVERSE SECTION OF A 27.8 MM. HUMAN EMBRYO (H 91, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 320

Differentiation of enamel organ and enamel knot of lower second premolar. *ek*—enamel knot. *iep*—inner layer of enamel epithelium. *lb*—lip-furrow band. *lieg*—lingual enamel groove. *oc*—oral cavity. *oep*—outer layer of enamel epithelium.

actively into the mesoderm, but grows centrifugally together with the surrounding tissues. The lip-furrow band is labial or buccal to the

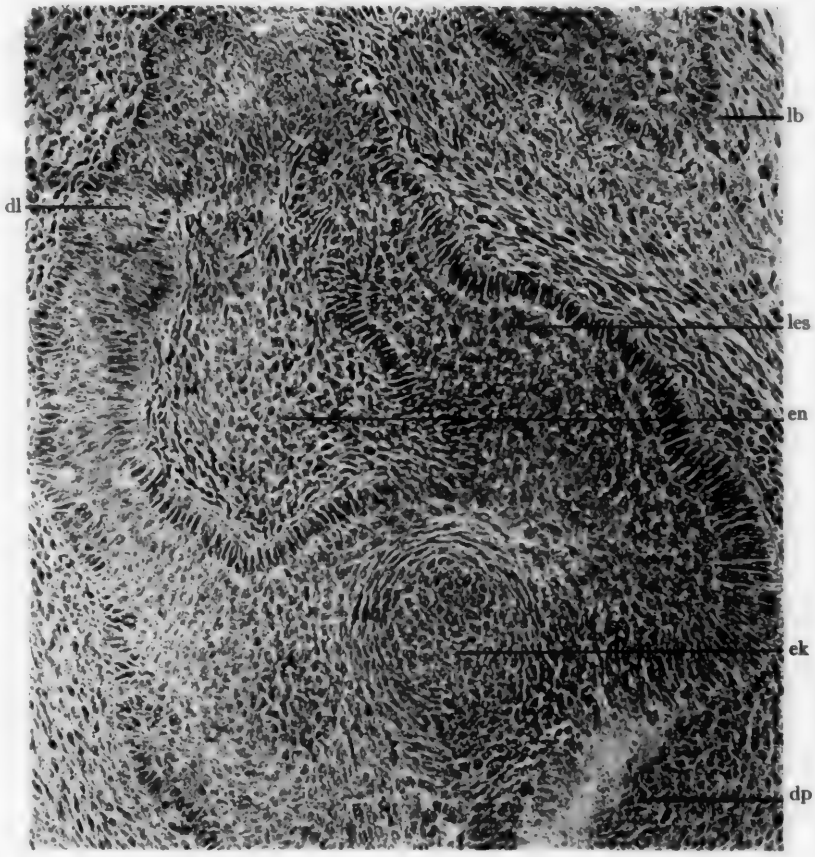

FIG. 4. TRANSVERSE SECTION OF A 60.4 MM. HUMAN EMBRYO (H 44, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 215

Tooth germ of lower cuspid showing enamel niche and lateral enamel strand. *dl*—dental lamina. *dp*—dental papilla. *ek*—enamel knot. *en*—enamel niche. *lb*—lip-furrow band. *les*—lateral enamel strand.

dental lamina. The bulb-like proliferations of the latter represent an early stage of the ectodermal portion of the tooth germs, and will soon become the enamel organ.

Dental papilla. A condensation of mesenchymal tissue is seen immediately below and around the deep end of the bulb, and represents the Anlage of the mesodermal portion of the tooth; and with further development becomes the dental papilla. The dental papilla develops into the pulp and dentine, and also contributes to the development of the cementum and peridental membrane.

Enamel organ. In Embryo H 91 (*fig. 3*) the epithelial bud has differentiated into the enamel organ. This structure now consists of an *outer* and *inner layer of enamel epithelium.* The inner layer, which will later form the enamel, is concave toward the mesenchyme, so that the enamel organ resembles the shape of a gastrula. Enclosed by the inner and outer layer is a central mass of large, round, or polyhedral cells. The cells at the center of the base of the enamel organ have become high columnar, and proliferated, and have arranged themselves to form a conical thickening extending chiefly into the enamel organ, but also projecting somewhat toward the dental papilla.

Enamel knot. Thus the general concavity of the inner tunic is interrupted at its center by a convex area. This structure was described by Ahrens as the enamel knot (*fig. 3*). It serves, according to Sicher and Tandler, as a germinal center for the rapidly growing enamel organ. *Fig. 4* shows a concentric arrangement of the cells of the enamel knot, and suggests a whirlpool of activity. However we did not observe any greater mitotic activity here than in other regions of the enamel organ. It is possible that we have here a migration of epithelial cells.

Labial and lingual enamel grooves. The projection of the enamel knot toward the dental papilla is at times bounded labially and lingually by what Ahrens calls the labial and lingual enamel grooves (*fig. 3*), which are believed to play an important part in the formation of the crown of the tooth.

Enamel cord. The enamel knot is connected with the summit of the enamel organ by a central epithelial extension, which Bolk calls the enamel septum. Since this structure is located only centrally, Ahrens calls it more appropriately the enamel cord (*fig. 5*). According to Schaffer, this enamel cord has a steadying effect on the central portion of the enamel organ, thus enabling it to maintain the invaginated condition of the latter. Bolk regards this cord as a remnant of

a partition that, in earlier development, divides the enamel organ into halves; and he employs it as evidence for his "dimer" theory. According to this theory, the teeth of the primates have a double origin, and have evolved through the fusion of two teeth.

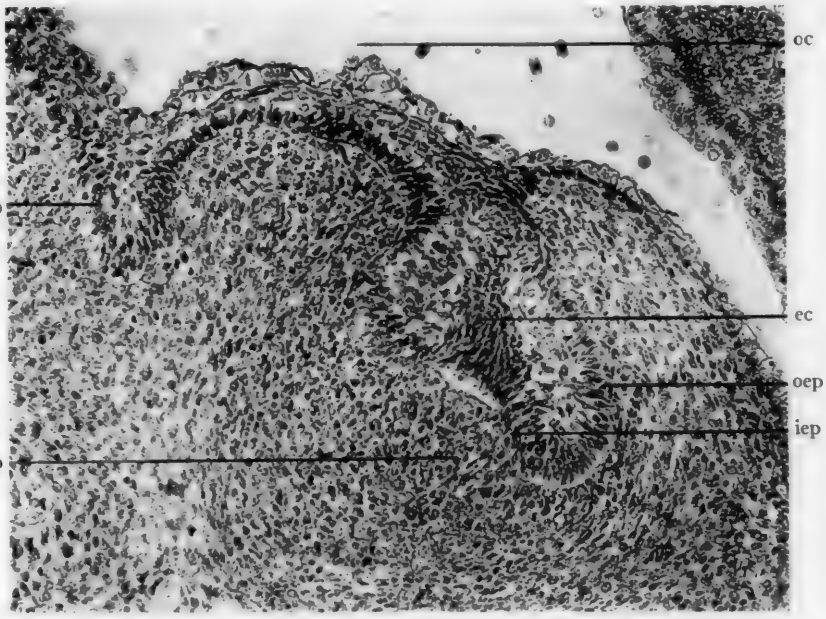

FIG. 5. TRANSVERSE SECTION OF A 27.8 MM. HUMAN EMBRYO (H 91, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 210

Differentiation of enamel organ and enamel cord of the lower second premolar. *dp*—dental papilla. *ec*—enamel cord. *iep*—inner layer of enamel epithelium. *lb*—lip-furrow band. *oc*—oral cavity. *oep*—outer layer of enamel epithelium.

Enamel navel. The enamel cord has been described as ending in a contraction at the summit of the enamel organ called the enamel navel. Both the enamel knot and the enamel navel disappear later.

Stellate reticulum. The polyhedral cells between the outer and inner layers of the enamel organ differentiate into stellate cells, arrange themselves in the form of a network, and thus form the stellate reticulum or the enamel pulp. A fully formed enamel organ for a lower

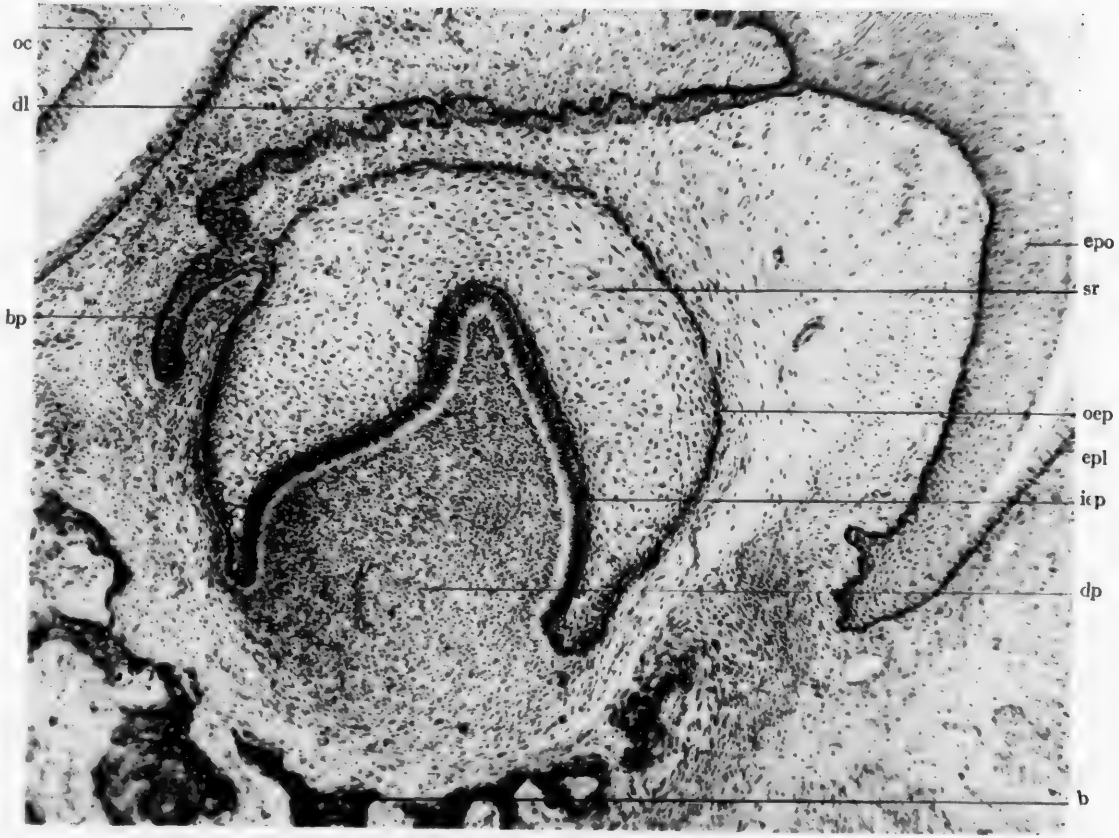

FIG. 6. SAGITTAL SECTION OF A LOWER CENTRAL INCISOR OF A FOUR-MONTH HUMAN EMBRYO (H 10, DEPARTMENT OF HISTOLOGY, COLLEGE OF DENTISTRY, UNIVERSITY OF ILLINOIS). MAGNIFICATION 80

b—bone of mandible. *bp*—bud for permanent tooth. *dl*—dental lamina. *epl*—epithelium of lip. *epo*—epithelium of oral cavity. *dp*—dental papilla. *iep*—inner layer of enamel epithelium. *oc*—oral cavity. *oep*—outer layer of enamel epithelium. *sr*—stellate reticulum. See *fig. 7.*

incisor of a human embryo is shown in *fig. 6*. It consists of the outer and inner layer of enamel epithelium and the stellate reticulum, and

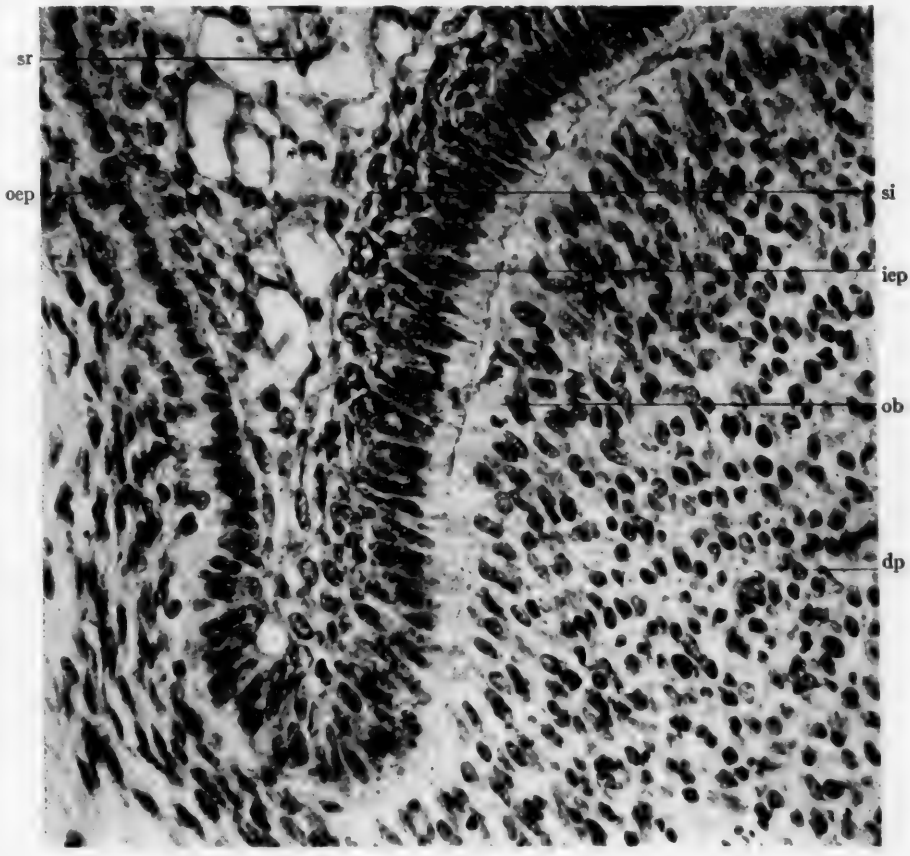

FIG. 7. PORTION OF *fig. 6*. MAGNIFICATION 640

dp—dental papilla. *iep*—inner layer of enamel epithelium. *ob*—future odontoblastic layer. *oep*—outer layer of enamel epithelium. *si*—stratum intermedium. *sr*—stratum reticulum.

covers the dental papilla. The inner layer is separated from the stellate reticulum by the *stratum intermedium*. (*Fig. 7*).

Lateral enamel strand. The enamel organ is connected with the

dental lamina by means of a broad attachment which, according to Sicher and Tandler, is soon split into two parts by the ingrowth of neighboring mesoderm. One part remains in immediate contact with the dental lamina. The other part is narrow. Because it connects the more lateral or labial portion of the enamel organ with the dental

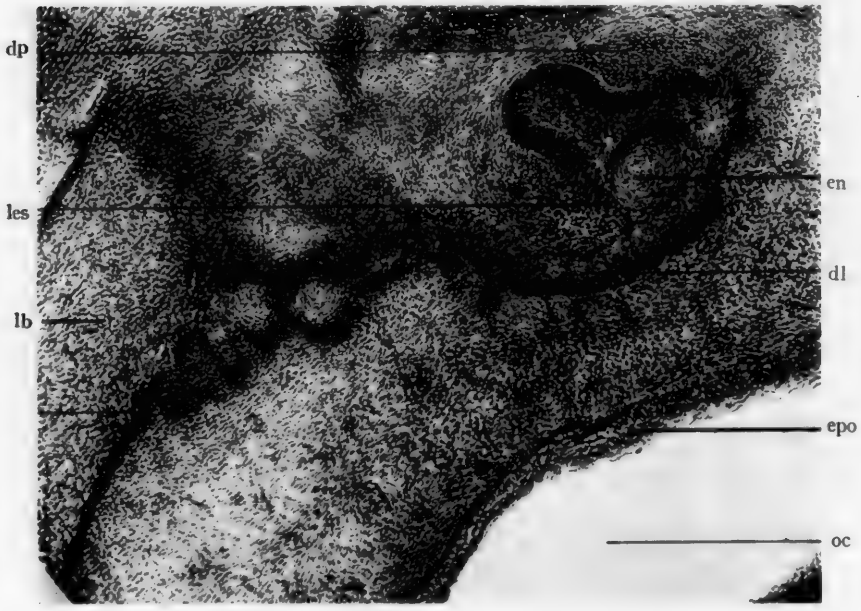

FIG. 8. TRANSVERSE SECTION OF A 57.5 MM. HUMAN EMBRYO (H 231, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 100

Tooth germ of upper lateral incisor, showing enamel niche and lateral enamel strand. *dl*—dental lamina. *dp*—dental papilla. *en*—enamel niche. *epo*—epithelium of oral cavity. *lb*—lip-furrow band. *les*—lateral enamel strand. *oc*—oral cavity.

lamina, Bolk has described it as the lateral enamel strand. He considers it the remnant of a second dental lamina from which arose a separate tooth germ corresponding to the labial portion of the enamel organ. In his opinion the dental lamina and the lateral enamel strand have an equal share in the formation of the enamel organ, which is thus a double structure.

Enamel niche. The space bounded by the dental lamina, the lateral enamel strand, and the enamel organ is called the enamel niche (Bolk). These relationships vary according to the stage of develop-

FIG. 9. TRANSVERSE SECTION OF A 57.5 MM. HUMAN EMBRYO (H 231, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 100

Tooth germ of lower cuspid, showing enamel niche and lateral enamel strand. *dl*—dental lamina. *dp*—dental papilla. *ek*—enamel knot. *en*—enamel niche. *epo*—epithelium of oral cavity. *iep*—inner layer of enamel epithelium. *lb*—lip-furrow band. *les*—lateral enamel strand. *oep*—outer layer of enamel epithelium.

ment and the type of tooth, and present different appearances according to the plane in which the sections are cut (*figs. 4, 8, 9*). Orban found that there are mesial and distal enamel niches.

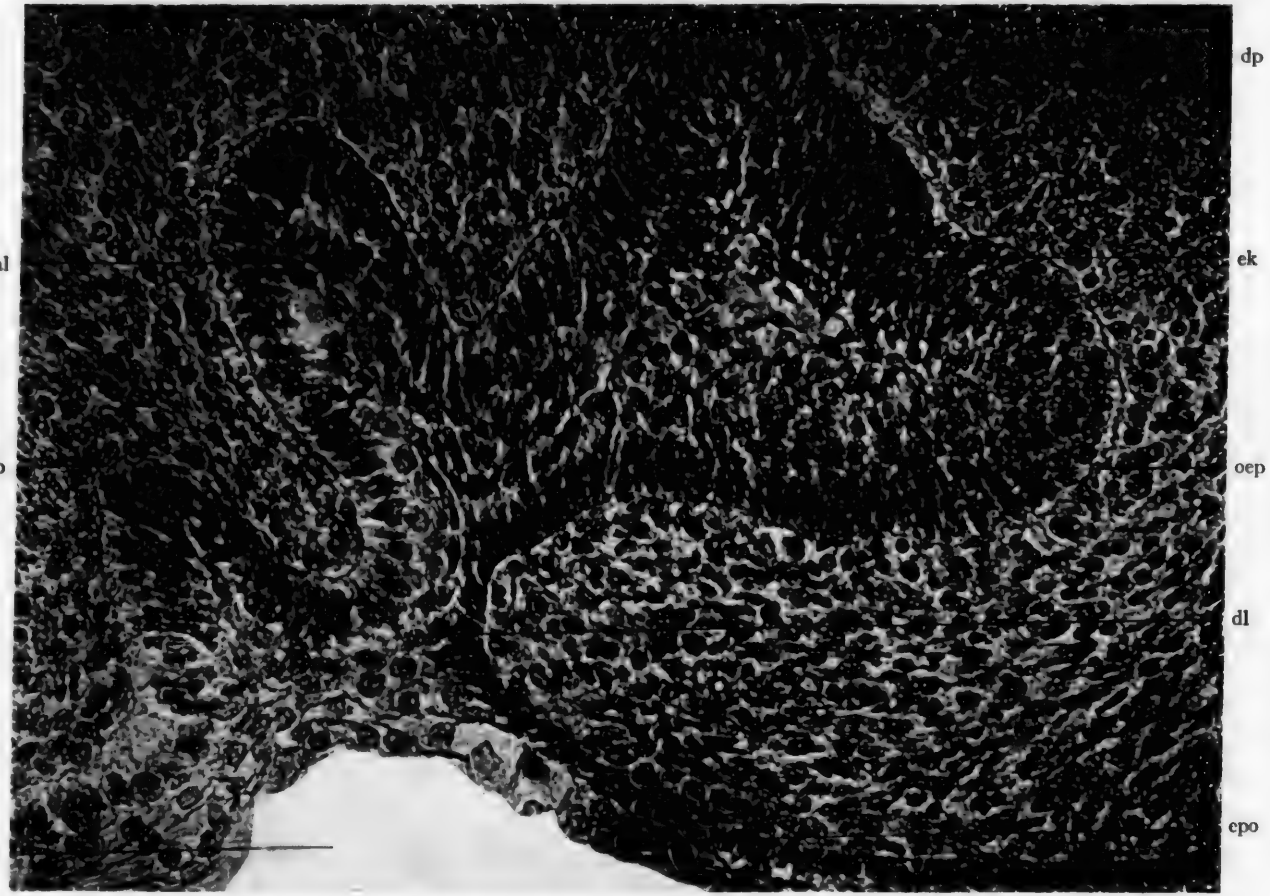

FIG. 10. TRANSVERSE SECTION OF A 27.8 MM. HUMAN EMBRYO. (H 91, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO).
MAGNIFICATION 425

Tooth germ of upper second premolar, with accessory lamina. *al*—accessory lamina. *dl*—dental lamina. *dp*—dental papilla. *ek*—enamel knot. *lb*—lip-furrow band. *oc*—oral cavity. *oep*—outer layer of enamel epithelium. *epo*—epithelium of oral cavity.

Epithelial Anlage of permanent tooth. As the enamel organ continues its growth, it assumes the shape of the future crown of the tooth and also determines the shape of the developing tooth (*fig. 6*). At the same time it loses its connection with the dental lamina, itself becoming free at its deeper margin. The free end is lingual to the tooth germ, and is significant because it represents the epithelial Anlage of the permanent tooth (*fig. 6*). This part of the dental lamina is called the secondary lamina, and extends downward and develops into the enamel organ for the corresponding permanent tooth. It passes through the same phases of development as are seen in the temporary teeth.

Fate of dental lamina. The dental lamina finally becomes absorbed, the process starting at the anterior end and proceeding posteriorly. Remnants of the lamina sometimes persist as epithelial pearls, and may differentiate into enamel masses or supernumerary teeth.

Accessory lamina. An accessory lamina is sometimes found labial to the dental lamina (*fig. 10*), sinking down into the mesoderm. It is probably a rudimentary structure, and is associated by various observers with the prelacteal tooth Anlagen, or the tooth glands found in reptiles. It disappears like the dental lamina. This structure presents an interesting field for further investigation.

II. THE RELATIONSHIP BETWEEN THE DENTAL LAMINA AND THE LIP-FURROW BAND

Development of dental lamina and lip-furrow band. The dental lamina appears earlier than the lip-furrow band, and grows more rapidly in the early stages of development. In later development, when the tooth germ has become independent of its attachment to the oral surface, the growth of the dental lamina is retarded while the lip-furrow band develops more rapidly, extends deeper, and becomes prominent (*fig. 6*).

Because of their close relationship in place of origin, there has been considerable controversy on the question of the development of the dental lamina and the lip-furrow band. According to Röse (1891) the primitive dental lamina appears in the thirty-fourth to the fortieth day, and later (17 mm. embryo) splits into two diverticula. The anterior diverticulum is more flattened and runs perpendicularly to

the Anlage of the lips. This diverticulum is associated with the formation of the lip-furrow band. The larger posterior diverticulum runs horizontal in a posterior direction, and forms the true dental lamina.

FIG. 11. TRANSVERSE SECTION OF A 27.8 MM. HUMAN EMBRYO (H 91, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 230

Tooth germ of lower cuspid. *dl*—dental lamina. *dp*—dental papilla. *epl*—epithelium of lip. *iep*—inner layer of enamel epithelium. *lb*—lip-furrow band. *oc*—oral cavity. *oep*—outer layer of enamel epithelium. *t*—tongue.

According to McMurrich (1912) the dental lamina, or the "dental ridge" as he calls it, does not differentiate into two parts but arises lingual to the "primary labial groove." In his view the latter and the dental lamina appear about the same time in embryos of about 11 mm. He recognizes that in the later stages the dental lamina "penetrates more deeply in the mesenchyme in a somewhat oblique direction," so that it appears as if it were derived from the "labial

FIG. 12. SECTION CUT PARALLEL TO FRONTAL BONE OF A 38.4 MM. HUMAN EMBRYO (H 98, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 25

Tooth germ of lower second premolar and dental lamina in region between lower first premolar and lower cuspid. *dl*—dental lamina. *lb*—lip-furrow band. *Mc*—Meckel's cartilage. *t*—tongue. *tg*—tooth germ.

groove." Schaffer (1922) states that during the second embryonic month the lamina, which runs along the margin of the jaws, splits in the region of the anterior teeth into a "distal" lip-furrow band and into a "medial" dental lamina; and that in the posterior region the lip-furrow band and the dental lamina arise independently, and a small distance apart. Orban (1928) states that the dental lamina and the lip-furrow band arise separately from the mouth epithelium, and that the dental lamina appears first. Our observations lead us to concur with McMurrich and Orban in their statements that the dental

lamina and the lip-furrow band arise independently, and do not develop by a splitting of the primary dental lamina.

Relationship between dental lamina and lip-furrow band in lower jaw. A study of serial sections of different embryos shows that in the lower jaw the relative distances between the dental lamina and the lip-furrow band decrease anteriorly. In the illustrations taken from

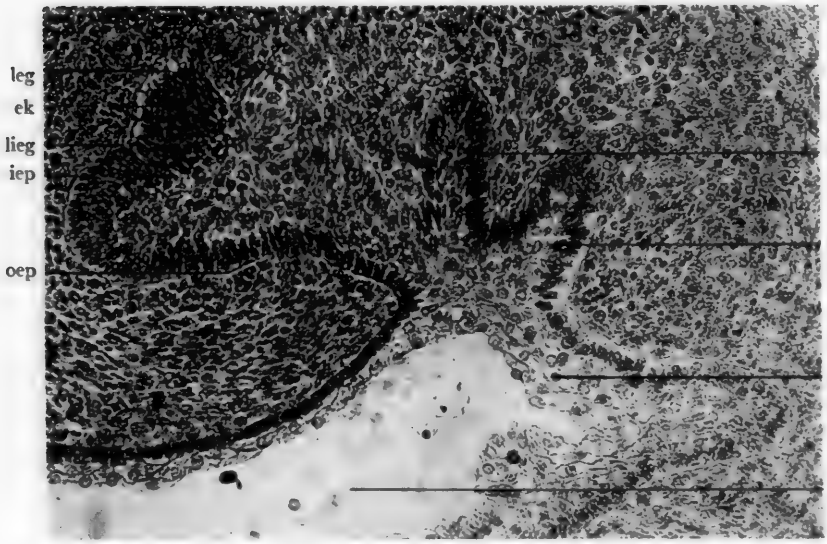

FIG. 13. TRANSVERSE SECTION OF A 27.8 MM. HUMAN EMBRYO (H 91, DEPARTMENT OF ANATOMY, UNIVERSITY OF CHICAGO). MAGNIFICATION 210

Tooth germ of upper second premolar with accessory lamina. *al*—accessory lamina. *ek*—enamel knot. *epo*—epithelium of oral cavity. *iep*—inner layer of enamel epithelium. *lb*—lip-furrow band. *leg*—labial enamel groove. *lieg*—lingual enamel groove. *oc*—oral cavity. *oep*—outer layer of enamel epithelium.

Embryo H 91, the distance between the dental lamina and the lip-furrow band is largest in *fig. 3*, smaller in *fig. 5*, and smallest in *fig. 11*, which is a more anterior section and in which the dental lamina and the lip-furrow band appear to have a common origin. At this point however the lip has developed and, probably because the latter has grown more rapidly than the dental lamina, a shifting and crowding of relationships occurred, resulting in this close approximation. *Fig.*

12, taken from Embryo H 98, shows a similar condition. This section is especially interesting because it was cut obliquely, so that it shows the tooth germ of the second premolar on the left side, and the dental lamina in the region between the first premolar and the cuspid on the right. On the left side, which represents a more posterior region, there is a considerable distance between the lip-furrow band and the tooth germ. On the left side the distance is smaller.

Throughout development and organogenesis in the growing embryo, irregularities in the forms of projections and invaginations are brought about by the condition that certain areas grow more rapidly than others. Concomitant with the workings of this law of unequal growth, there occurs a considerable amount of changing and shifting of spacial relationships. Likewise, concomitant with the further differentiation of the free margins of the jaws and with the more rapid development in the direction of lip formation taking place previous to the formation of the cheeks, there is at first a greater crowding of relationships in the more anterior region, which results in the apparent common origin of the dental lamina and the lip-furrow band as seen in *fig. 11*.

Relationship between dental lamina and lip-furrow band in upper jaw. When we observe in the same embryo the spacial relationship between the dental lamina and the lip-furrow band in the upper jaw, we find that they are everywhere nearer to each other than in the lower jaw (*figs. 10, 13*). This can also be explained by the workings of the laws of unequal growth and interdependence of developmental processes. The development of the face is influenced considerably by the growth of the brain, which precedes it in development and which controls to some extent its lateral and sagittal dimensions, so that the face is low and broad. At the same time the face develops in the service of the differentiation of the sense organs, and of the nasal and oral cavities. Among the various structures that are to develop within the upper portion of the face, the upper teeth start relatively later and develop relatively slower. The more rapid growth and development of the other structures within a limited amount of space result in a lateral shifting and crowding, so that among various effects the arch of the upper jaw projects beyond that of the lower, and the dental lamina and the lip-furrow band are brought nearer to each other.

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ANNOUNCEMENTS

1. DANGEROUS TOOTH CLEANING FLUIDS

"Taxi" (Holland Specialty Co., Peoria, Ill.)

and

"Stain Remover" (Ransom and Randolph Co., Toledo, Ohio)

Advertised as non-injurious tooth-cleaning fluids, although rapidly destructive of enamel. See the findings of the Research Council of the New York Academy of Dentistry, and the comment by the manufacturers, in the *Journal of Dental Research*, 1927, vii, pp. 477–500.

2. LYMEOGENE: a harmful dentifrice

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